SHOUT
SHARE
IT

LEATHER, DRES

JAN-1967

P
11
Botany

9

89612
Smith

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief
University of Wisconsin

WILLIAM CROCKER,
The Thompson Institute For Plant Research, Inc.

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

H. H. BARTLETT,
University of Michigan

ARTHUR H. GRAVES,
Brooklyn Botanic Garden

I. F. LEWIS,
University of Virginia

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

VOLUME IX—1922

WITH THIRTY-FIVE PLATES AND ONE HUNDRED AND FOURTEEN TEXT FIGURES

SMITHSONIAN INSTITUTION
283600
U. S. NATIONAL MUSEUM

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT PRINCE AND LEMON STREETS, LANCASTER, PA.

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

TABLE OF CONTENTS, VOLUME IX, 1922

No. 1, January

Page

Ecological relations of plants in southeastern Missouri (with three text figures and Plates I and II) . . . . J. C. Th. Uphof 1

Methods of healing in some algal cells (with Plate III) Susan P. Nichols 18

The present status of Scolopendrium in New York State (with two text figures) . . . . Mabel R. Hunter 28

Biophysics as a point of view in plant physiology Howard E. Pulling 37

No. 2, February

The growth of field corn as affected by iron and aluminum salts (with six text figures and Plate IV) . . . . Charles Homer Arndt 47

Control of the sexual state in Arisaema triphyllum and Arisaema Dracontium . . . . John H. Schaffner 72

A demonstration of numerous distinct strains within the nominal species Pestalozzia Guepini Desm. . . Carl D. La Rue and H. H. Bartlett 79

No. 3, March

Undercooling of peach buds (with one text figure) . . Earl S. Johnston 93

Significance of the behavior of sensitive stigmas . . . . . F. C. Newcombe 99

The relation of age to size in certain root cells and in vein-islets of the leaves of Salix nigra Marsh. (with three text figures) Marjorie A. Tellefsen 121

The origin of new varieties of Nephrolepis by orthogenetic saltation II. Regressive variation or reversion from the primary and secondary sports of bostoniensis (with Plates V–X) . . . . R. C. Benedict 140

No. 4, April

Hydrogen-ion concentration in its relation to wheat scab (with 18 text figures) . . . . E. F. Hopkins 159

On the physiological balance in nutrient solutions for plant cultures W. F. Gericke 180

Vegetative vigor of the host as a factor influencing susceptibility and resistance to certain rust diseases of the higher plants I. M. A. Raines 183

Rots of early strawberries in Florida and southern California (with four text figures) . . . . Neil E. Stevens 204

No. 5, May

Page

Potato ovules with two embryo sacs (with one text figure) W. J. Young 213
Vegetative vigor of the host as a factor influencing susceptibility and resistance to certain rust diseases of the higher plants II (with Plates XI and XII) . . . . M. A. Raines 215
Lignification of mature phloem in herbaceous types (with Plate XIII) Carl L. Wilson 239
Observations on the effect of water-raking on the keeping quality of cranberries . . . . H. F. Bergman 245
Incipient drying and wilting as indicated by movements of coconut pinnae (with one text figure) . . . . Sam F. Trelease 253
Dr. W. J. Beal's seed-viability experiment . . . . H. T. Darlington 266
Nutrient solutions for wheat . . W. E. Tottingham and E. J. Rankin 270

No. 6, June

Comparative studies on respiration XXI. Acid formation and decreased production of CO_2 due to ethyl alcohol (with two text figures) . . . . Marian Irwin and Margaret Weinstein 277
A new species of Spirogyra with unusual arrangement of the chromatophores (with three text figures) . . . . Mabel L. Merriman 283
The male receptacle and antheridium of Reboulia hemisphaerica (with 24 text figures and Plate XIV) . . . . A. W. Dupler 285
The meiotic cytokinesis of Nelumbo (with one text figure and Plate XV) . . . . Clifford H. Farr 296
Comparative studies on respiration XXII. The effect of lactic acid on the respiration of wheat (with two text figures) Edith Philip Smith 307
The effect of transpiration on the absorption of salts by plants Walter C. Muenscher 311
Prairie inclusions in the deciduous forest climax (with two text figures) Herbert C. Hanson 330

No. 7, July

A preliminary study of Claviceps purpurea in culture (with Plates XVI–XXI) . . . . Walter W. Bonns 339
Studies in the genus Gymnosporangium IV. Distribution of the mycelium and the subcuticular origin of the telium in G. clavipes (with seven text figures and Plate XXII) . . . . B. O. Dodge 354
Certain relations between root development and tillering in wheat: significance in the production of high-protein wheat W. F. Gericke 366
The pollination of Marcgravia: a classical case of ornithophily? (with five text figures and Plates XXIII and XXIV) . . Irving W. Bailey 370

PAGE
Environmental temperatures of fungi in nature (with one text figure) NEIL E. STEVENS 385
The reduction divisions in the pollen mother cells of Oenothera franciscana (with Plates XXV–XXVII)........RALPH E. CLELAND 391

No. 8, OCTOBER

Studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in seed plants.............................THOMAS WYATT TURNER 415
A preliminary notice of genetical studies of resistance to mildew in Oenothera..........PETER J. KLAPHAAK AND H. H. BARTLETT 446
Growth of some parasitic fungi in synthetic culture media (with four text figures)..............H. C. YOUNG AND C. W. BENNETT 459

No. 9, NOVEMBER

The structure of the starch grain (with nine text figures) O. L. SPONSLER 471
A study of the white heart-rot of locust, caused by Trametes robiniophila (with three text figures) C. H. KAUFFMAN AND H. M. KERBER 493
The origin and development of lamellae in Agaricus campestris and in certain species of Coprinus (with 12 text figures and Plates XXVIII–XXXV)..........................MICHAEL LEVINE 509

No. 10, DECEMBER

The effect of Bordeaux mixture upon the chlorophyll content of the primordial leaves of the common bean, Phaseolus vulgaris L. W. A. RUTH 535
The effect of low-temperature storage and freezing on fruits and vegetables...............................LON A. HAWKINS 551
Revision of the South American species of Cuscuta I. T. G. YUNCKER 557
Index to Volume IX.. 576

(Dates of publication: No. 1, Feb. 21; No. 2, Mar. 20; No. 3, Apr. 24; No. 4, May 15; No. 5, June 21; No. 6, July 12; No. 7, Aug. 30; No. 8. Oct. 20; No. 9, Nov. 27; No. 10, Jan. 8, 1923.)

ERRATA, VOLUME IX

Page 150. Line 19 should read:
also in the greenhouses of Peter Wagner of Brooklyn.[5]
Page 188, line 32; and page 193, line 1. *Puccinia Caryophylli* should be *Uromyces caryophyllinus.*
Page 323. In line 8, *soulution* should be *solution.*
" 342. In lines 26 and 27, *of a parasite* should be *or a parasite.*
" 464. In next to last line of table, *Colletotricum* should be *Colletotrichum.*

CORRECTION

Through an oversight, the writer failed to state in his paper, "Studies of the Mechanism of the Physiological Effects of Certain Mineral Salts in Altering the Ratio of Top Growth to Root Growth in Seed Plants" (pp. 415–445), that the method used in growing isolated root tips was first suggested by Dr. W. J. Robbins, who had previously demonstrated its practicability.

Thomas W. Turner

Vol. IX. JANUARY, 1922 No. 1

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

CONTENTS

Ecological relations of plants in southeastern Missouri . . . . J. C. TH. UPHOF 1
Methods of healing in some algal cells . . . . SUSAN P. NICHOLS 18
The present status of Scolopendrium in New York State . . MABEL R. HUNTER 28
Biophysics as a point of view in plant physiology . . . . HOWARD E. PULLING 37

Smithsonian Institution
FEB 25 1922
National Museum

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879.

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
The Thompson Institute For Plant Research, Inc.

H. H. BARTLETT,
University of Michigan

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ARTHUR H. GRAVES,
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

JACOB R. SCHRAMM,
Cornell University

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Postage to all foreign countries, 20 cents a volume on annual subscriptions. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume, post free.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Ffty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

FEB 25 1922
National Museum

AMERICAN
JOURNAL OF BOTANY

VOL. IX JANUARY, 1922 NO. 1

ECOLOGICAL RELATIONS OF PLANTS IN SOUTHEASTERN MISSOURI

J. C. TH. UPHOF

(Received for publication April 26, 1921)

The state of Missouri can be divided physiographically into three distinct regions: (1) the prairie region or glacial plain in the northwest and a prairie region in the middle west; (2) the Ozark region toward the south, southwest, and partly southeast; and (3) the lowlands in the southeastern corner.

The writer has made his botanical observations in the southeastern part of the state; to which belong a large part of the Ozark uplift and the lowlands in the extreme southeast. Counties which were mainly studied are Madison, Iron, Reynolds, Wayne, St. Francois, Bollinger, Carter, Ripley, Butler, where an important part of the Ozark Mountains is located, and Butler, Stoddard, Scott, Mississippi, New Madrid, Dunklin, and Pemiscot, where most of the lowlands are found.

The plant growth of this section of the state has hardly been studied; therefore no data of other investigators could be examined with the exception of those who explored other parts of Missouri such as Mackenzie (4), Daniels (2), and Hus (3). Only Bush (1) made some collections in this part of the state, and published a list of specimens found.

The author is much indebted for the kind help he received from Mr. C. A. Gierth of Poplar Bluff, Mo.

Excursions were made by the writer in 1918 throughout the entire spring and summer; the various plant associations in relation to their environment were studied, with the exception of those which are the result of the activities of mankind, such as the plant growth along canals, railroads, on cultivated farms, and so on.

The Ozark region, as it appears in the counties above named and in others not mentioned here, is composed not of an arrangement of mountain chains but of short ridges and rounded peaks with numerous valleys. The highest peaks in this part are to be found in Iron County, attaining a height of 540 meters; in this county occur the greatest variations in altitude of the entire Ozark Mountains; in St. Francois County the highest elevation is about 330 meters; whereas the highest hill in Butler County is about 200 meters above sea level.

[The **Journal** for December (471-533) was issued Feb. 15, 1922.

Geologically the Ozarks are complex; crystalline rocks are to be found in the southeastern part of the state, namely in the St. Francois Mountains, which are considered oldest. Further, several beds of limestone and sandstone are to be met. The various systems are either Carboniferous, Devonian, Silurian, Ordovician, Cambro-Ordovician, or Cambrian.

Erosion has played an important part in the topography of the country; therefore soil types are of various kinds and to a considerable extent derived from the various rocks in their respective environment. The physical properties of these soils greatly influence the development and the differentiation of the flora; they are grey to reddish-brown in color, contain but little organic matter, often stones are present, and rock outcrops are very common. Level areas are the least stony as a rule. The reddish-brown soils are usually derived from cherty limestones of the lower Carboniferous group, the grey soils derived from the Cambro-Ordovician group being silicious limestones. The oxidation of iron gave to the soil a reddish-brown color, which is absent in grey soils of the cherty limestones. The character of the soil varies from a stony to a silt loam.

Many streams and creeks traverse the country and are the cause of constant erosion, giving to a large extent the present aspect of the topography of the Ozarks. Outside the rainy periods they are harmless little streams, often entirely dry during the largest part of the summer; after heavy rains, or sometimes after sudden melting of a heavy snowfall, they grow to wide streams, overflowing the surrounding country and denuding areas of a part of the soil and vegetation, and often giving a different aspect to the environment.

The climate of the southeastern part of Missouri is characterized by hot summers and mild winters. The temperature data which the writer was able to obtain are given in table 1.

TABLE 1. *Temperatures in Southeastern Missouri*

Month	Mean	Maximum	Minimum
	° C.	° C.	° C.
January	− 1.11	+ 3.33	− 5
February	+ 0.55	5.55	− 3.33
March	8.89	13.30	+ 2.78
April	15	20.55	10.55
May	18.89	25	13.89
June	25	31.10	20.55
July	31.67	40.55	24.44
August	31.11	38.89	23.30
September	24.44	31.67	20
October	16.10	22.22	11.10
November	8.89	16.11	3.85
December	5	10	− 0.55

In the higher portions of the mountain ridges and peaks the temperature is lower. Considerable cold spells are of short duration; in some winters there is no snowfall. The average snowfall amounts to 275 mm. in the southeast, as compared with 600 mm. in the northwestern part of the state.

The atmosphere is very humid and depressing. The rainfall in southeastern Missouri is higher than that in any other part of the state. Although no exact data could be obtained, the following were derived from sources of the United States Weather Bureau: 800 to 950 mm. in parts of northern Missouri, 950 to 1,100 mm. in the middle section, 1,100 to 1,500 mm. in the southeast being the mean annual rainfall over 21 years. During the summer rainless periods may occur of 2 to 6 weeks' duration; whereas sudden, heavy rainfalls accompanied by thunderstorms may occur at any time during the summer; these rains swell the rivers suddenly and cause them to overflow the surrounding country, especially the lowlands.

The mean growing period of the vegetation lasts from March to October.

Plant associations are very much diversified on account of their localities, such as small river valleys, ravines, barrens, mountain summits, rock outcrops, and small prairies; each one has its own plant societies. Frequently species are encountered belonging to southern states which are not to be found in the northern or middle part of the state.

The forests of the Ozark hills are a predominant feature, being entirely different in composition from those of the lowlands. The soil of these hills is poor in organic matter as far as the slopes are concerned; the reddish-brown or grey soil, composed of very fine particles, is everywhere visible; when wet it is very sticky, when dry it is baked and very hard. Various oak species are here in the majority; although in a certain area one species of oak may be more frequently observed than in any other part of the hills. Toward the southern portion, *e.g.*, in Butler County, *Quercus marilandica* Muench. forms a considerable part of the forest growth; in richer uplands *Quercus imbricaria* Michx. is often present; other species which are never absent are *Q. stellata* Wang. ; *Q. falcata* Michx. ; *Q. macrocarpa* Michx.; *Q. alba* L.; *Q. rubra* L.; hybrids of *Q. alba* × *Q. macrocarpa* and of *Q. rubra* × *Q. falcata* are also sometimes observed. The heaviest trees are always referable to *Q. alba*, *Q. macrocarpa*, and *Q. rubra.*

Other genera of trees met with in such dry hills are, in the first place, several hickories, especially *Carya glabra* (Mill.) Spach, *C. ovata* (Mill.) K. Koch; where the soil is more fertile big trees of *C. alba* (L.) K. Koch also become common. Further, *Diospyros virginiana* L., *Liquidambar Styraciflua* L., *Sassafras officinale* Nees & Eberm., and *Nyssa sylvatica* Marsh. occur on such sterile soils; they become, however, far more common when the soil is fertile. Of smaller kinds of trees one notices especially in the spring before the leaves are unfolded the early flowering *Cercis canadensis* L., although it is as abundant in the rich bottom lands and ravines; at this time of the year the trees are covered with the beautiful pink-colored flowers; *Cornus florida* L. also grows in practically the same localities. On the other hand, *Ulmus alata* Michx., a species with conspicuously winged twigs, belongs decidedly to the dry, sterile uplands of the above mentioned counties.

The composition of these forests as far as the various species of trees are concerned is expressed in table 2, the result of surveying typical hills densely covered by woods in a few counties.

Table 2

Name of Tree	A Forest in Western Butler County %	A Forest in Middle Iron County %	A Forest in Middle Wayne County %	A Forest in Southern Madison County %	Diameter of Thickest Tree Observed (dm.)
Quercus marilandica Muench	25	6	15	—	3
" *imbricaria* Michx	0.3	20	—	—	8
" *alba* L.	10	2	—	1	9
" *rubra* L.	8	4	6	—	8
" *macrocarpa* Michx	23	10	10	30	12
" *falcata* Michx	14	15	—	12	6
Carya glabra (Mill.) Spach	11	6	4	12	5
" *ovata* (Mill.) Koch	—	—	2	8	6
" *alba* (L.) Koch	—	4	6	—	6
Juglans nigra L.	—	—	2	—	9
" *cinerea* L.	—	3	6	8	6
Ulmus alata Michx	5	2	8	2	4
Sassafras officinale Nees & Eberm	—	4	15	16	8
Diospyros virginiana L.	2	15	12	6	3
Liquidambar Styraciflua L.	1	8	11	—	7
Fraxinus americana L.	0.7	1	—	—	7
Nyssa sylvatica Marsh	some	—	—	2	8
Cornus florida L.	some	some	2	1	1½
Cercis canadensis L.	some	some	1	2	1

Of all these species *Quercus marilandica* is the most typically xerophytic species and especially adapted to dry barrens; where the hills are becoming fertile, as toward the base of the hill near a stream, this oak species disappears completely.

The shrub vegetation is not always heavily developed between the high trees, but is common toward the edge of the woods. Here are frequently found very extensive thickets of *Symphoricarpos orbiculatus* Moench covering several acres in extent, often forming a pure association where nothing else is found until the forests are entered, where the shrubs become gradually less abundant. Other species of shrubs and small trees which may grow either in pure stands or in a mixed growth are *Rhus typhina* L., *R. canadensis* Marsh., *Ribes gracile* Michx.; whole patches are covered by *Ceanothus americanus* L. and *Rosa* species, especially *R. blanda* Ait. and *R. pratincola* Greene, whereas in other areas *R. humulis* Marsh. is prevalent. In various hills of the above described type the slopes are entirely covered by *Vaccinium melanocarpum* Mohr extending into the Quercus facies, although in the latter places they do not grow so densely; they are especially common in the hills of the western portion of Butler County and in Wayne County;

although they occur also in other parts. In the southwestern part of Butler County and in the neighborhood of Poplar Bluff in the same county, several individuals were found of *Vaccinium arboreum* Marsh., of which the highest plant attained a height of 3½ meters; the highest ones I met in this region grew in a scattered wood especially where *Quercus marilandica*, *Q. macrocarpa*, and *Carya ovata* were predominating trees in a sandy loam.

In spring the woods are covered with several early flowering plants, being to a large extent bulb- or rootstock-bearing species; whole areas form a dense association of *Podophyllum peltatum* L. where hardly anything else is able to find a place. Along other parts of such woods there are, either scattered or in small patches, *Anemonella thalictroides* (L.) Spach, *Viola pedata* L., *Oxalis violacea* L., and *Hypoxis hirsuta* (L.) Coville. These species are common where trees are less dense or in open parts of the woods, places which are very exposed to drought; but the rootstocks and bulbs of these species are well protected. The thick roots of Anemonella, containing reserve food, are surrounded by a tissue of cork; whereas the bulbs of Oxalis and Hypoxis are protected by a fibrous mass consisting of the remains of scales. Where the soil contains more moisture and humus, interesting growths of *Claytonia virginica* L., *Delphinium tricorne* Michx., and *Viola palmata* L. are to be noticed. The undergrowth of these woods and thickets is later, in the early summer, succeeded in the first place by large areas of *Potentilla canadensis* L. which are especially a feature of situations having a rather sandy subsoil. Where this species is not present the surface is practically everywhere covered by *Houstonia coerulea* L. which flowers until midsummer. Elsewhere, however, plenty of space is given to other species, where all those mentioned with the exception of few Podophyllums and Potentilla are growing; to which may be further added *Krigia Dandelion* (L.) Nutt., *Fragaria virginiana* Duchesne, *Geum canadense* Jacq., and *Erigeron vernus* (L.) T. & G. With the exception of Fragaria these species practically never cover considerable areas, and therefore one cannot speak of a certain association. When woods are becoming somewhat exposed, such species as *Monarda Bradburiana* Beck, *Silene virginica* L., *Sisyrinchium campestre* Bicknell, and considerable areas of *Antennaria plantaginifolia* (L.) Rich. abound.

Later in the year the early flowering species gradually disappear. Houstonia is to a large extent replaced by *Stylosanthes biflora* (L.) B.S.P., in other sections by *Lechea minor* L. or *Cunila Mariana* L. Pure stands of each of the latter may cover one hill after the other. At this time of the year (from July to September) long rainless intervals often have much influence on the vegetation of these hills, for which the above named species are well adapted. On very exposed slopes only very drought-resistant species are to be noticed, among which *Euphorbia maculata* L., *Agave virginica* L., *Croton capitatus* Michx., and *C. monanthogynus* Michx. are commonest. Where plant growth is to a certain extent protected by the shade of only a few big trees or by slopes toward eastern and northern exposures, a well developed growth can be observed of a large number of perennial plants, most of which are composites such as *Solidago rigida* L., *S. Drummondii* T. & G., *Aster turbinellus* Lindl., *A. patens* Ait., *Helianthus occidentalis* Riddell, *H. petiolaris* Nutt., *H. orgyalis* DC., *Lepachys pinnata* (Vent.) T. & G., *L. columnaris* (Sims) T. & G., *Ambrosia bidentata* Michx., *A. psilostachya* DC., *Silphium laciniatum* L., *Elephantopus carolinianus* Willd., *Vernonia crinita* Raf., *V. altissima* Nutt., *Rudbeckia triloba* L., and the very beautiful *Echinacea purpurea* DC. Species of other families which are often found here are *Monarda fistulosa* L., *M. mollis* L., *M. punctata* L., *M. citriodora* Cerv. (rare), *Pycnanthemum flexuosum* (Walt.) B.S.P., *P. pilosum* Nutt., *Scutellaria canescens* Nutt., *S. pilosa* Michx., *Pentstemon Cobaea* Nutt., *P. hirsutus* (L.) Willd., *Gerardia grandiflora* Benth. (often between oaks), *G. flava* L., *Linum virginianum* L., *Euphorbia corollata* L., *E. heterophylla* L. (rare in Butler County), *Apocynum androsaemifolium* L., *A. cannabinum* L., *Asclepias tuberosa* L. (rare), *A. purpurascens* L., *A. quadrifolia* Jacq., *A. verticillata* L. (occasionally), *Amsonia Tabernaemontana* Walt.; also *Gaura coccinea* Pursh, *Cuphea petiolata* (L.) Koehne, *Tephrosia virginiana* (L.) Pers., *Baptisia bracteata* (Muhl.) Ekl., and *Pteris aquilina* L. Several of these species are also to be found on treeless barrens, and some of them are common on the prairie-like areas. A semi-parasite growing on the roots of oaks, *Comandra umbellata* (L.) Nutt., is very common in the entire region.

All these perennial plants usually form a dense vegetation, where a struggle for maintenance seems to exist, although there do not appear to be large areas covered only by one or two species.

Where a little creek passes through the dry hills, although it may be dry during the greater part of the summer, different vegetation is always found to have established itself. The soil contains more organic matter, and on account of the humus it contains and keeps its moisture longer and better than the surrounding loam. In such areas one finds, besides the already mentioned spring flora, several other interesting species. *Oakesia sessiliflora* (L.) Wats. forms whole groups; the creeping *Asarum canadense* L. covers whole patches, also *Pedicularis canadensis* L., *Heuchera hirsuticaulis* (Wheelock) Rydb., and *Mitella diphylla* L.; whereas *Trillium recurvatum* Beck and the early flowering annual *Collinsia verna* Nutt. are always to be met with. These species are all to be seen flowering in the spring and until early in summer, but later in the season they are gradually followed by *Lobelia inflata* L., *Silene stellata* (L.) Ait. f., *Spigelia marilandica* L., *Tradescantia virginiana* L., *T. rosea* Vent., and a few species of ferns, also such species as we find in the ravines and little valleys.

The climbing plants are not as well represented as they are in the valleys or in the rich lowlands. Very conspicuous here are two species of Passiflora, namely *P. incarnata* L., with large flesh-colored flowers, and *P. lutea* L., with small pale to greenish-yellow flowers. The former species is commoner in the southern counties, whereas the latter is further distributed toward the north. They grow either around twigs and stems of shrubs and young trees or are to be found without any support on treeless areas. Also *Vitis rupestris* Scheele grows sometimes in similar places.

Descending the above-described types of barren hills, the plant growth becomes different, mainly for two reasons: (1) a higher water content of the soil; and (2) a larger percentage of organic matter in the soil, which has partly accumulated after heavy rains from higher parts of the hills.

Tree growth is here entirely different; oaks like *Quercus marilandica* have disappeared, and are replaced by *Q. alba* and *Q. rubra; Juglans nigra* L. is here commoner; and other new species come to the front, such as *Betula lenta* L., *B. nigra* L., *Carpinus caroliniana* Walt., *Ostrya virginiana* (Mill.) K. Koch. Where a little valley is reached through which a little stream flows the number of various species of trees becomes high; it can not be said that a certain species predominates, as they form a mixture of *Populus heterophylla* L., *P. deltoides* Marsh., *Alnus incana* (L.) Moench, *Salix nigra* Marsh., and *Platanus occidentalis* L. on very wet and fertile soil; whereas further back these are accompanied by *Celtis occidentalis* L., *C. mississippiensis* Bosc., *Morus rubra* L., *Gleditsia triacanthos* L., *Pyrus coronaria* Mill., *Ulmus racemosa* Thom., *Gymnocladus canadensis* Lam. *Cercis canadensis* L., *Crataegus* sp., *Prunus serotina* Ehrh., and *Maclura aurantiaca* Nutt. (especially common in the southern counties). Impenetrable thickets composed of many species of shrubs and young trees are here practically always present; they are frequently composed of whole groups of *Corylus americana* Walt., *Sambucus canadensis* L., *Hydrangea arborescens* L., *Rhamnus lanceolata* Pursh, *Itea virginica* L., and *Physocarpus opulifolius* (L.) Maxim. In the more southern counties, such as Butler and Ripley counties, one finds in similar localities species which belong to the southern part of the state, among which I mention in the first place *Neviusia alabamensis* Gray, which the writer found in a single individual about 12 kilometers west of Poplar Bluff growing in a southeastern exposure on a somewhat sandy-loam slope of a small hill near the bank of a little creek; previously this species has been found only in a few localities in the state of Alabama. *Decumaria barbara* L. has been found along the bank of a little stream (Davis Creek) in Carter County, in but three individuals. Along such creeks and little river valleys there are a great many different kinds of climbing plants, especially *Ampelopsis quinquefolia* L., *Cissus stans* Pers., *Vitis cinerea* Engelm., *V. riparia* Michx., *V. aestivalis* Michx., *Rhus Toxicodendron* L., *Tecoma radicans* (L.) Juss., and *Menispermum canadense* L. Along the lower parts of the slopes and near creek beds is *Gelsemium sempervirens* (L.) Ait. f., a little vine of the southern states which sometimes occurs in some parts of Butler and Ripley counties; on the other hand, I was never able to find it in the more northern counties such as Iron and Madison. Of herbaceous and annual climbers, *Ipomoea pandurata* (L.) Mey., both Passifloras, *Sicyos angulatus* L., *Echinocystis lobata* (Michx.) T. & G., *Humulus Lupulus* L., and *Smilax herbacea* L. are common.

The spring flora is in such places well represented by various species of different families; besides the plants already mentioned from the forests of the hills (*Oxalis violacea* and *Hypoxis hirsuta* excepted), there are present along moist places *Cardamine bulbosa* (Schreb.) B.S.P., *Ranunculus septentrionalis* Poir., *R. fascicularis* Muhl., *Viola blanda* Willd., *Sanguinaria canadensis* L., *Peltandra virginica* (L.) Kunth, and *Dicentra Cucullaria* (L.) Bernh.; and *Mertensia virginica* (L.) Link not seldom occurs in large patches. Later, in the early summer, flower *Hydrastis canadensis* L., *Stylophorum diphyllum* (Michx.) Nutt. (rare), *Hybanthus concolor* (Forster) Spreng., *Dodecatheon Meadia* L., *Anemone virginiana* L., *A. pennsylvanica* L., and rarely *Cypripedium candidum* Muhl. and *Pogonia trianthophora* (Sw.) B.S.P. In the summer and autumn the ground is covered by a pure association of *Impatiens fulva* Nutt., with but a very few other species growing in between. Where Impatiens is absent there is usually found a diversified flora composed of *Cardamine pennsylvanica* Muhl., *Iris versicolor* L., *Laportea canadensis* (L.) Gaud., *Boehmeria cylindrica* (L.) Sw., *Arisaema triphyllum* (L.) Schott, and *A. Dracontium* (L.) Schott. Pteridophytes are well represented by *Adiantum pedatum* L., *Phegopteris hexagonoptera* (Michx.) Fée, *Asplenium acrostichoides* Sw., *Onoclea sensibilis* L., *Osmunda Claytoniana* L., *Botrychium virginianum* (L.) Sw., and sometimes *Ophioglossum vulgatum* L. In Butler County and other southern counties *Hymenocallis occidentalis* (Le Conte) Kunth, with its bright, beautiful white flowers, can frequently be observed along small river valleys.

During the latter part of the summer one finds, besides the nettle association of Boehmeria and Laportea, scattered almost everywhere, *Campanula americana* L., *Scrophularia marilandica* L., *Parietaria pennsylvanica* Muhl., *Circaea lutetiana* L., *Hydrophyllum canadense* L., *Muhlenbergia mexicana* (L.) Trin., *M. racemosa* (Michx.) B.S.P., *Uniola latifolia* Michx., *Diarrhena diandra* (Michx.) Wood, and several groups of *Eupatorium urticaefolium* Reich. and *Solidago latifolia* L.

In some of the counties the author has observed woods of *Pinus echinata* Mill. Once these were very common and extensive, but practically all have been cut down by lumber companies; only very little has been left. The writer found west of Carter County a small forest where this species of pine formed practically the entire growth under the trees. Here and there the small-shrub vegetation is composed of little thickets or single individuals of *Symphoricarpos orbiculatus* Moench, *Ceanothus americanus* L., and in open places of the woods of a single *Rhus typhina* L., whereas very large areas are entirely shrubless. The general aspect of the areas covered with *Pinus echinata* is generally that of dry, stony lands.

Where limestone outcrops occur along considerable areas in the mountains, *Juniperus virginiana* L. usually covers large parts and forms pure stands. In Iron County these conifers sometimes are very common, growing toward the summits of the mountains and along the edges of the cliffs. Lower down, *Celtis occidentalis* L., *Acer Negundo* L., *Amelanchier canadensis* (L.) Medic., *Fraxinus quadrangulata* Michx., *Morus rubra* L., *Quercus marilandica* Muench., *Q. Muhlenbergii* Engelm., *Diospyros virginiana* L., *Carya alba* (L.) Koch, and, under these, the shrubs *Cornus asperifolia* Michx., *Viburnum rufidulum* Raf., and *Hydrangea arborescens* L. are always present. On treeless portions of such rock outcrops the plant growth is very much exposed to extreme heat and drought; therefore the flora is often xerophytic or semi-xerophytic, except near dripping ledges and little springs; here *Selaginella rupestris* (L.) Link, *Woodsia obtusa* (Spreng.) Torr., *Cystopteris fragilis* (L.) Bernh., *Aquilegia canadensis* L., *Solidago Drummondii* T. & G., and *Heuchera hispida* Pursh are predominating; in places less rich in soil *Pellaea atropurpurea* (L.) Link and *Cheilanthes Feei* Moore usually are the commonest species; on rock outcrops, somewhat shaded, in Ripley County, the writer found many plants of *Asplenium viride* Huds. and *Pellaea dealbata* Car. and a few individuals of the southwestern *Pellaea mucronata* DC.; on the highest elevations of such cliffs and bare mountain summits, *Draba cuneifolia* Nutt., *D. brachycarpa* Nutt., and *Androsace occidentalis* Pursh are prevalent. On flat places *Astragalus distortus* T. & G., and *Scutellaria parvula* Michx. are very common. Where the situation is shadowed by some tree or by rocks and is somewhat moist, but nevertheless stony and covered with very little soil, a dense association of *Camptosorus rhizophyllus* (L.) Link, the well-known walking fern, covers several rocks, growing from one stone to the other and in this way sometimes covering considerable areas. Between rocks, *Cystopteris bulbifera* (L.) Bernh., *Notholaena dealbata* (Pursh) Kunze, *Woodsia obtusa* (Spreng.) Torr., *Polystichum acrostichoides* (Michx.) Schott, *Asplenium platyneuron* (L.) Oakes, *A. Bradleyi* DC. Eaton (rare), *Erigeron philadelphicus* L., *Arabis dentata* T. & G., and *Opuntia Rafinesquii* Engelm. occur almost everywhere.

Where the rocky aspect gradually disappears and more soil accumulation is to be seen, such species occur as *Desmanthus illinoensis* (Michx.) MacM., *Schrankia uncinata* Willd., *Cassia Medsgeri* Shafer, *C. nictitans* L., *Agave virginica* L., *Cooperia Drummondii* Herb., *Eryngium yuccifolium* Michx., *Verbascum Thapsus* L., *Desmodium paniculatum* (L.) DC., *D. marilandicum* (L.) DC., *Psoralea tenuiflora* Pursh, and practically all composites which are to be found in the above-described hills of the oak facies.

The prairie flora is not here well represented; although sometimes barren, treeless areas appear several square kilometers in extent. They occur in almost any of the counties in the Ozarks as far as they were studied. The principal vegetation is composed of grasses, especially *Agropyron spicatum* Scribn., *Bouteloua oligostachya* (Nutt.) Torr., *B. curtipendula*

(Michx.) Torr., and *Panicum* sp.; further, *Liatris squarrosa* Willd., *L. scariosa* Willd., *Petalostemum purpureum* (Vent.) Rydb., *Euphorbia corollata* L., *Croton monanthogynus* Michx., *Astragalus mexicanus* A. DC., and especially *Trifolium stoloniferum* Muhl. and *Plantago aristata* Michx. predominate; like the true prairies, these barren places have no vernal flora.

This description of the plant societies and associations of the Ozark uplift of the southeastern portion of Missouri gives a general idea of the nature of its flora.

The lowlands, covering about 7,500 square kilometers and lying about 300 feet above sea level, are largely composed of residue from the Mississippi River and its tributaries, such as the Black River, St. Francois River, and others. These bottom lands are composed of various types of very fertile loam soils. They are complex in origin, as the various rivers obtained their material from such sources as the residual uplands, the plains, the loessial and glacial prairies; consequently they differ from one another in composition and color, ranging from sandy loam to silty loam. The soils west of Crowley's Ridge, representing the old valley of the Mississipp River, are very old; usually they are grey in color; through poor drainage and constant leaching a considerable loss of lime, iron, and sometimes of phosphorus has resulted. A silty loam of a great depth is the principal soil of this region; it includes most of the land between the upland and the western part of Crowley's Ridge. The northern part, however, consists of recent alluvium, derived from the uplands, and contains at various places much organic matter. The subsoil usually is a drab silty clay.

West of Crowley's Ridge, in Stoddard County and Ripley County, a relatively large area is found with fine sandy loam; it is greyish brown to yellowish grey, whereas the subsoil is composed of sandy clay; it contains many iron concretions, which may result in hardpan formation. This country stands three to eight decimeters above the surrounding lowlands.

From about Cape Girardeau County throughout the lowlands toward the boundaries of Arkansas, the soil is heavy and contains much organic matter, whereas the subsoil is of a close structure. These areas are the lowest of any part of southeastern Missouri, which fact has resulted in the formation of swamps of considerable extent.

These swamps are of extreme interest to the ecologist; many formations and associations are here to be encountered, starting in the floating plant associations of Lemnas and ending in the strange swamps with Taxodium and monotonous Nyssa forests.

Following the various facies of the different plant associations, one may readily distinguish the stagnant pools and ponds, the surface of the water thickly covered by floating plants most of which belong to the Lemnaceae; a pond is covered either by a single species or by several. The following are found: *Wolffia punctata* Griseb., *W. papulifera* C. H. Thomps. (rare), *Wolffiella floridana* (J. D. Sm.) Thomps., *Spirodela polyrhyza* (L.) Schleid., *Lemna valdiviana* Phil., *L. perpusilla* Torr., and *L. minor* L. *Azolla caro-*

liniana Willd. is also very common in quiet or almost stagnant waters, so covering the surface of the water as to give the appearance of a large lawn. *Riccia natans* L. is frequently to be found but is never as common as the other species belonging to the floating flora. These species are, apart from several algae, the first appearance of any vegetation in these watery areas.

About seven meters below the water surface there are a few species of plants rooting in the mud; a very few individuals may here be found of *Isoetes Engelmanni* A. Br.; between the depths of five and seven meters a number of submerged species are prevalent. Where the water is clear, large areas can be noticed of "meadows" with *Elodea canadensis* Michx.; where this growth is not as dense, *Potamogeton Hillii* Morong, *P. pusillus* L., and *Najas flexilis* (Willd.) Rostk. & Schmidt are present; and occasionally *Ceratophyllum demersum* L. and some *Vallisneria spiralis* L. In a few ponds I found whole areas filled with *Myriophyllum heterophyllum* Michx., and nothing else. In slowly flowing streams and little rivers, *Batrachium aquatilis* L., and especially *Heteranthera graminea* Vahl, are very common; the Black River is for several miles covered with the latter species.

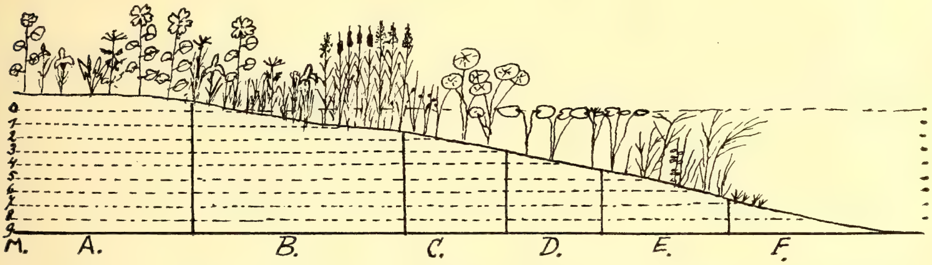


FIG. 1. A swamp formation. *A*, Hibiscus, Sium, Cicuta, Rumex, Iris, Sagittaria, Mimulus, Lobelia. *B*, the same, and Phragmites, Typha, Acorus, Sparganium. *C*, Scirpus, Nelumbium. *D*, Nymphaea, Nuphar. *E*, *Potamogeton Hillii*, Najas, Elodea, Ceratophyllum, Isoetes. *F*, Isoetes.

The facies of the water lilies and their associates are formed at a depth of from two to four meters below the surface; these *aquatilis natantes* form dense masses of *Nymphaea odorata* Ait. and *Nuphar advena* Ait. Commonly found in these places are *Potamogeton hybridus* Michx., *P. americanus* C. & S., and *P. natans* L.; where the growth of water lilies is too dense the Potamogetons are unable to grow. Frequently they form pure associations without any other water plant. Between these are often found the above-described submerged species; whereas *Isoetes Engelmanni* becomes more frequent, *I. melanopoda* J. Gay is also noticed.

Toward the border of the lake the water-lily association includes considerable tracts of *Nelumbo lutea* (Willd.) Pers. often reaching the border of the lake and large pools. They may be considered as forming an intermediate between the floating water plants and the amphibious vegetation; they root in the mud from 1½ to 3 meters below the surface of the water. Here also several forerunners are growing of *Pontederia cordata* L. Taking these pure aquatic plants as a whole, there are relatively few species in comparison with the amphibious and semi-amphibious species along the edges of the lakes.

The margins of the ponds and lakes are surrounded by a great number of different species of amphibious plants; sometimes they form pure associations, but mostly they occur in societies of different species. From a depth of ½ to 1 meter the lakes are thickly covered with *Scirpus lacustris* L., *S. fluviatilis* (Torr.) Gray (rare), *Typha latifolia* L., *Phragmites communis* L., *Sagittaria lancifolia* L., *S. graminea* Michx., *Acorus Calamus* L., *Zizania palustris* L., *Glyceria fluitans* (L.) R. Br. (also sometimes between the water lilies), *G. nervata* (Willd.) Trin., *Dulichium arundinaceum* (L.) Britton (rare), *Iris fulva* Ker, and *I. hexagona* Walt. In less pure associations are to be seen *Alisma Plantago* L., *Sparganium eurycarpum* Engelm., *S. lucidum* Fern. & Eames; *Nelumbo lutea* also often grows here, although in few individuals. A very interesting species growing in ponds, borders of lakes, and even on mud flats, is *Thalia dealbata* Roscoe, a member of the Marantaceae which reaches here in this region one of its northern limits. Frequently it is associated with the beautiful flowering *Hymenocallis occidentalis* (Le Conte) Kunth. Mud flats and shallow ponds are for several miles covered with *Polygonum Muhlenbergii* (Meisn.) Wats., hardly showing any other species in their association; their rootstocks are very deeply imbedded in the mud. Also *Cicuta maculata* L., *Saururus cernuus* L., and *Jussiaea diffusa* Forst. are growing amongst such mud plants. When the mud flats become partly dry in summer there may be for a time a vegetation of any species whose seeds happen to drop and to germinate there, but these always disappear when the land becomes inundated again. To such species belong *Polygonum Hydropiper* L., *Solanum carolinense* L., *Humulus alatus* Ait., *Lippia lanceolata* Michx., *Rotala ramosior* (L.) Koehne, and a few others. When such mud flats happen to remain dry, these and other species cover the surface entirely; then a struggle for life soon eradicates some of the least resistant individuals, and eventually entire species.

Other amphibious plants along the margins of lakes are *Equisetum hyemale* L., *Cicuta maculata* L., *Sium cicutaefolium* Schrank, *Rumex crispus* L., *R. verticillatus* L., *Jussiaea diffusa* Forst., *Bacopa rotundifolia* (Michx.) Wettst., *Senecio aureus* L., *Carex monile* Tuckerm., *C. conjuncta* Boott, *Mimulus ringens* L., *Stachys palustris* L., *Diodia virginiana* L., *Ranunculus abortivus* L., *Ludvigia palustris* (L.) Ell., *Lobelia siphilitica* L., *L. cardinalis* L., *Nasturtium palustre* L., and (in large tracts) *Hibiscus lasiocarpus* Cav.

Very large areas of swamps are covered with *Taxodium distichum* (L.) Richard and *Nyssa aquatica* L. Gigantic trees of the former as well of the latter form pure stands or grow mixed near one another covering several square kilometers in extent. The Taxodium forest has only few associates especially in deep swamps; they grow successfully only when moisture is abundant. Taxodium is "the" tree for alluvial swamps. Lands which are overflowed regularly by rivers are especially suitable for this species; only in the dry seasons do a part of these forested mud fields become dry. The trees develop a straight stem; where water is deep, the base of these stems is always broad and more or less ridged; where water is more or less shallow, or in localities not as much subject to heavy overflow, this base is not as broad or ridged and is normally developed like that of ordinary trees. A peculiar characteristic of these trees consists of the development of knees, vertical outgrowths of the superficial root system, which give a strange aspect to the physiognomy of this plant association. Knees occur only where the ground is covered for a long time or all the year round by water. These knees attain a height of from 4 to 15 decimeters; very occasionally they are higher. According to various authors they serve the purpose of supplying oxygen to the root system in the mud, as well as strengthening the support of the tree on the surface.

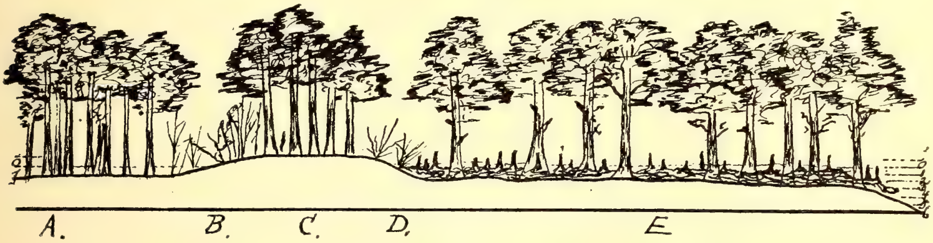


FIG. 2. A swamp forest. *A*, *Nyssa sylvatica*. *B*, *Salix nigra*. *C*, *Acer rubrum*, *Quercus alba*, *Q. rubra*, *Platanus occidentalis*, etc. *D*, *Cephalanthus occidentalis*. *E*, *Taxodium distichum*.

Another representative of the swamps which is frequently associated with Taxodium is *Nyssa aquatica* L. A pure forest tree of medium age shows a peculiar aspect on account of the straight, smooth stems; hundreds of such trees have the appearance of as many straight pillars. Old individuals attain a height of from 18 to 28 meters. The base of old stems is thickly covered by mosses and by a small fern, *Polypodium polypodioides* (L.) Hitchc. Where Taxodium swamps are not too deep there exists an immense undergrowth of *Polygonum Muhlenbergii* (Meisn.) Wats. On outstanding mud flats or in open shallow places of the Taxodium forest, *Cephalanthus occidentalis* L. is of common occurrence, forming dense thickets. Sometimes a single *Salix nigra* Marsh., or rarely *Dirca palustris* L., occurs; in some swamps the author observed a dense growth of *Leitneria floridana* Chapm. in places similar to those occupied by Cephalanthus, while on logs *Itea virginica* L. often occurs.

Where the swamp becomes shallow, or where it is even dry during a large part of the season, a greater variation in species of trees and shrubs at once manifests itself. Taxodium and Nyssa become scarcer, and a mixed stand appears of *Acer rubrum* var. *Drummondii* (H. & A.) T. & G., *Planera aquatica* (Walt.) J. F. Gmel., *Populus deltoides* Marsh., *P. heterophylla* L., *Carya aquatica* (Michx. f.) Nutt., *Fraxinus profunda* Bush., *Styrax americana* Lam., and *Gleditsia aquatica* Marsh.; the latter sometimes forms pure, impenetrable stands. Frequently there occur here treeless areas which are usually covered with *Juncus acuminatus* Michx., *J. scirpoides* Lam., *Eleocharis acuminata* (Muhl.) Nees, *E. intermedia* (Muhl.) Schultes, *E. palustris* (L.) R. & S., *Cyperus flavescens* L., and *C. acuminatus* Torr. & Hook., besides most species which are also to be found along the treeless margins of the lake. In some old mud ponds where a part of the year water has been left, characteristic groups of *Salix nigra* Marsh. and *S. longifolia* Muhl. cover large stretches.

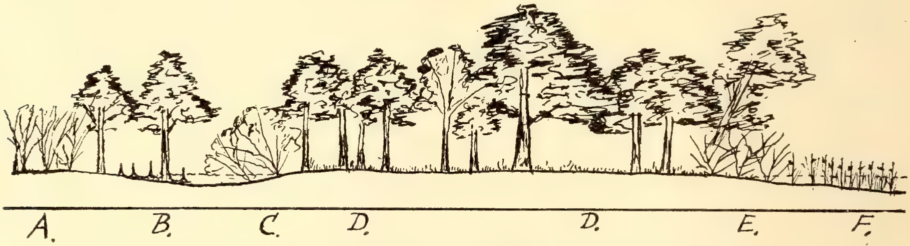


FIG. 3. A mixed forest. *A*, *Leitneria floridana*. *B*, *Taxodium distichum*. *C*, *Salix nigra*. *D*, mixed growth of *Platanus occidentalis*, *Morus rubra*, *Celtis occidentalis*, *Quercus* sp., *Ulmus americana*, etc. *E*, *Cephalanthus occidentalis*, *Sambucus canadensis*. *F*, swamp with *Scirpus lacustris*.

On the higher elevations of the bottom lands which are not as often subject to overflow, with the exception of high floods caused by such streams as the Black River and the St. Francois River, dense forests are to be found of a very diversified tree growth. On account of the richness of the soil the trees attain considerable height and thickness, one of the largest being *Platanus occidentalis* L., which is followed in size by beautiful individuals of *Quercus alba* L., *Q. rubra* L., *Q. macrocarpa* Michx., *Q. bicolor* Willd., *Q. Michauxii* Nutt., *Ulmus fulva* Michx., *U. americana* L., *Tilia americana* L., *Juglans nigra* L., *J. cinerea* L., *Sassafras officinale* Nees & Eberm., as well as of *Quercus phellos* L., *Morus rubra* L., *Celtis occidentalis* L., *C. mississippiensis* Bosc., *Liquidambar Styraciflua* L., *Acer rubrum* (H. & A.) T. & G., *Fraxinus americana* L., *Carya alba* (L.) Koch, *C. illinoensis* (Wang.) K. Koch, *Pyrus coronaria* L., *Aesculus Pavia* L., *Gymnocladus canadensis* Lam., *Cercis canadensis* L., *Magnolia tripetala* L., *Liriodendron Tulipifera* L., *Cladrastis lutea* (Michx. f.) Koch, *Crataegus* sp., *Populus heterophylla* Marsh., and *P. deltoides* Marsh. All these species grow near one another, not one forming a pure stand with the exception of *Asimina triloba* Dunal, which often covers whole thickets several acres in extent. On such lands Taxodium and Nyssa are very rare.

The author has endeavored to give in tabular form (table 3) the number of trees of certain species of a few areas in these bottom lands, also the diameter of the thickest trees he found in this region.

TABLE 3

Name of Tree	A Forest in Dunklin County %	A Forest in Butler County %	A Forest in Butler County %	A Forest in St. Francois County %	Diameter of Thickest Tree Observed (dm.)
Taxodium distichum (L.) Rich.	100	—	76	2	31
Nyssa aquatica L.	—	100	24	1	15
Acer rubrum var. *Drummondii* (H. & A.) T. & G.	—	—	—	8	8
Platanus occidentalis L.	—	—	—	6	22
Quercus alba L.	—	—	—	11	12.5
Q. rubra L.	—	—	—	4	11
Q. phellos L.	—	—	—	2	5
Q. Michauxii Nutt.	—	—	—	1.5	15
Juglans nigra L.	—	—	—	2	12
J. cinerea L.	—	—	—	1	8
Carya alba (L.) Koch	—	—	—	4	7
C. illinoensis (Waug.) K. Koch	—	—	—	1	9
Morus rubra L.	—	—	—	2.5	6
Tilia americana L.	—	—	—	0.5	8
Liriodendron Tulipifera L.	—	—	—	0.5	14
Fraxinus americana L.	—	—	—	2	11
Celtis occidentalis L.	—	—	—	3	4
Ulmus americana L.	—	—	—	2.5	22
Salix nigra Marsh	—	—	—	20	9
Liquidambar Styraciflua L.	—	—	—	5	12
Planera aquatica (Walt.) J. F. Gmel.	—	—	—	2	2
Populus heterophylla L.	—	—	—	3	8
Leitneria floridana Chapm.	—	—	—	5	0.4
Gymnocladus canadensis Lam.	—	—	—	0.5	4
Asimina triloba Dunal	—	—	—	10	2.5

The Vitis association is very pronounced in such moist forests and along river banks. Dense and dark entanglements through which it is difficult to penetrate are formed by *Vitis cordifolia* Michx., *V. riparia* Michx., *V. cinerea* Engelm., *Ampelopsis quinquefolia* Michx., and *A. cordata* Michx.; to this list may be added *Menispermum canadense* L., *Cocculus carolinus* (L.) DC., *Celastrus scandens* L., *Rhus Toxicodendron* L., *Tecoma radicans* (L.) Juss., *Bignonia capreolata* L., *Aristolochia macrophylla* Lam., *Lonicera dioica* L., *Wisteria macrostachya* Nutt., and *Clematis virginiana* L. Some of these reach the summits of the high trees, and attract much attention during the flowering period. Herbaceous climbers are here represented by *Smilax herbacea* L., *S. rotundifolia* L., *S. Bona-nox* L., *Dioscorea villosa* L., *Passiflora incarnata* L., and *Sicyos angulatus* L.

Where forest trees make up a dense formation there are but few species of shrubs; but along the edges of these woods and where trees are more scattered there are several species of shrubs and small trees, the principal ones which are found being *Carpinus caroliniana* Walt., *Ostrya virginiana*

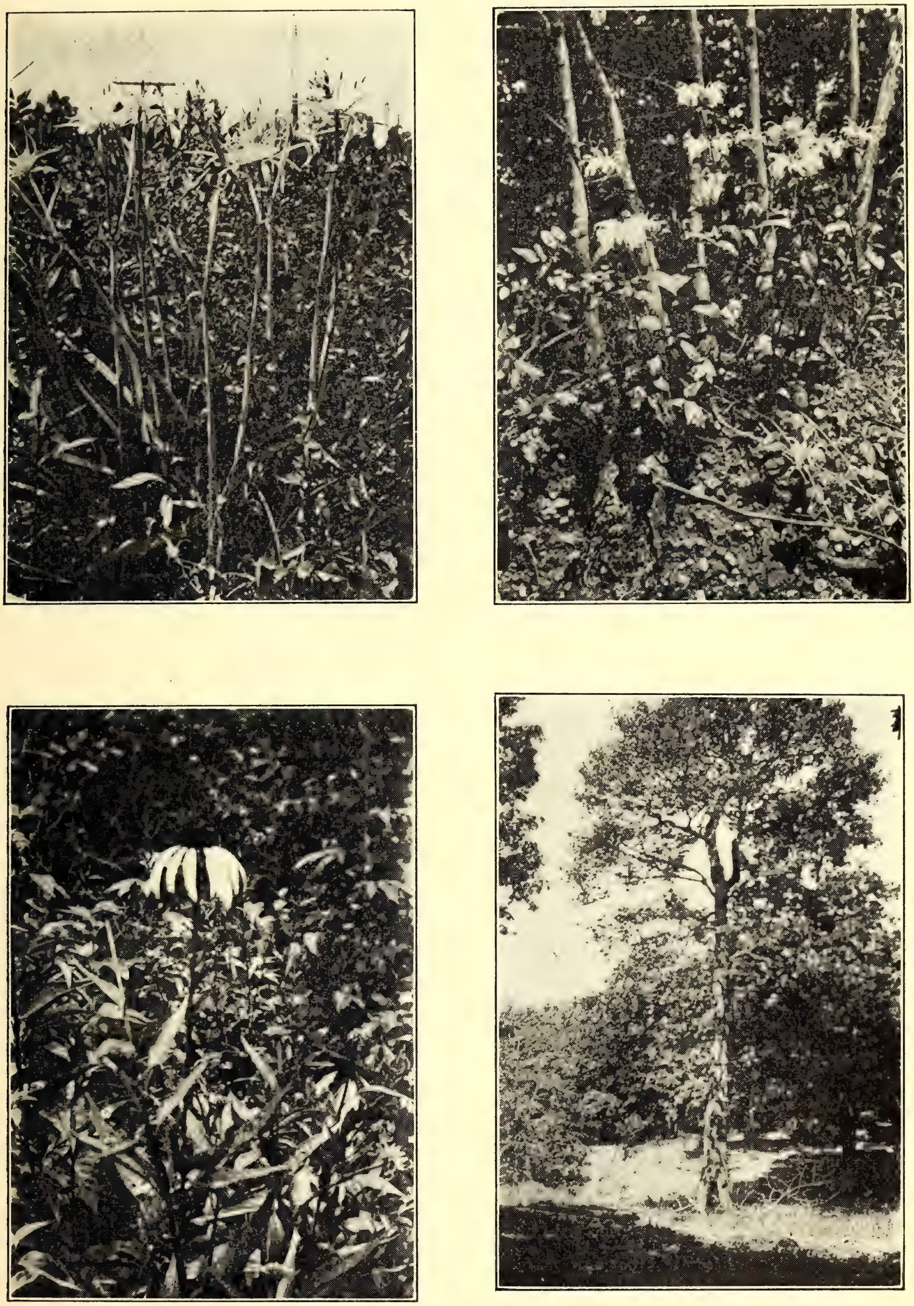
(Mill.) K. Koch, *Ptelea trifoliata* L., *Ilex decidua* Walt., *I. opaca* Ait., *I. Cassine* L., *Cephalanthus occidentalis* L., *Staphylea trifolia* L., *Xanthoxylum americanum* Mill., *X. Clava-Herculis* L. (rare), *Sambucus canadensis* L., *Corylus americana* Walt., and *C. rostrata* Ait. (both forming dense thickets), *Cornus stolonifera* Michx., *C. florida* L., *Hamamelis virginiana* L., *Adelia acuminata* Michx., *Styrax americana* L., *Lindera Benzoin* Blume, *Physocarpus opulifolius* (L.) Maxim., *Ribes gracile* Michx., *Rosa carolina* L., *Amorpha fruticosa* L., *Cercis canadensis* L., *Chionanthus virginica* L., *Viburnum prunifolium* L., and *Amelanchier canadensis* (L.) Medic.

In the spring and early summer the woods are extremely attractive during the flowering period of such plants as Magnolia, Cercis, Chionanthus, Adelia, Cornus, and Asimina.

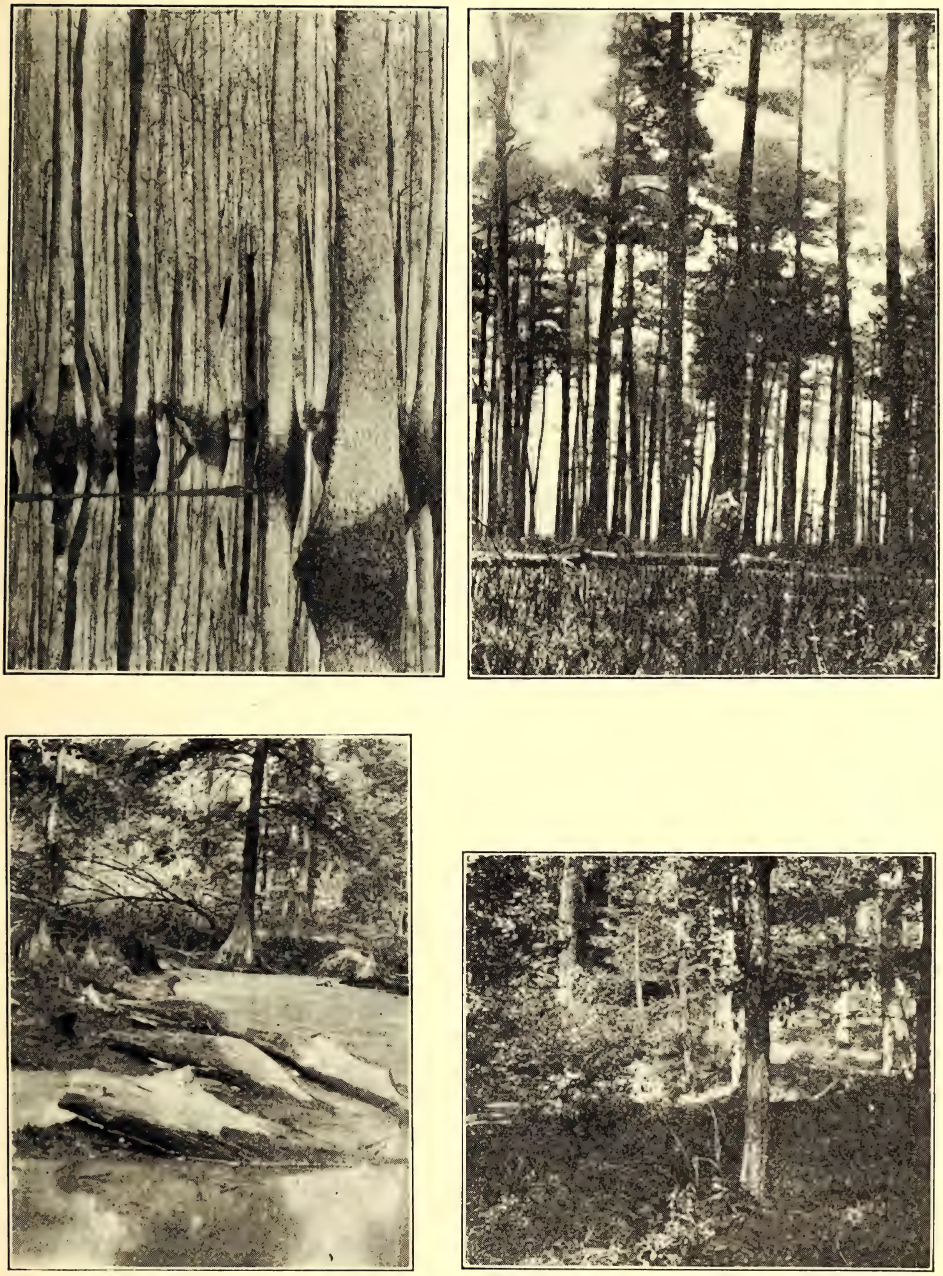
During the spring several perennial plants flower. One of the earliest is *Symplocarpus foetidus* (L.) Nutt., of which the spadix at first appears and later in summer the broad, veiny leaves. *Phlox divaricata* L. shows its bright blue flowers in almost any part of the woods. *Mertensia virginica* (L.) Link frequently covers large patches between the trees. Other vernal plants which are of common occurrence are *Claytonia virginica* L., *Uvularia grandiflora* Sm. (rare), *Erythronium americanum* Ker, *E. albidum* Nutt., *Trillium recurvatum* Beck, *T. grandiflorum* (Michx.) Salisb. (rare), *Dodecatheon Meadia* L., *Trientalis americana* (Pers.) Pursh, *Podophyllum peltatum* L., *Thalictrum purpurascens* L., *Viola blanda* Willd., *Arisaema triphyllum* L., and *Hydrastis canadensis* L.

During summer one finds in these dense forests extensive areas covered with *Boehmeria cylindrica* (L.) Sw., *Pilea pumila* (L.) Gray, and *Laportea canadensis* (L.) Gaud., forming the nettle association. In less dense areas there is a diversified perennial vegetation of *Rudbeckia laciniata* L., *Eupatorium coelestinum* L., *E. urticaefolium* Reich., *E. perfoliatum* L., *Elephantopus carolinianus* Willd., and clumps of the beautiful flowering *Spigelia marilandica* L.; the bright scarlet flowers of *Lobelia cardinalis* L. are visible at a long distance. Between the various herbs creeps *Eryngium prostratum* Nutt., while at some places *Commelina virginica* L. is very common. Tracts of land which are denuded of trees are covered with *Actinomeris squarrosa* Nutt. Of other common species I will mention *Silphium perfoliatum* L., *Ambrosia trifida* L., *Lactuca ludoviciana* (Nutt.) Riddell, *Botrychium virginianum* (L.) Sw., *B. ternatum* (Thunb.) Sw. and *Adiantum pedatum* L.

The bottom lands along the Mississippi in the southeastern section of the state are to a large extent covered by big woods of the same type, mixture, and character as those described above in the swamps and somewhat higher lands; marshes are here common. Directly along the shores are dense stands of *Phragmites communis* L., *Cyperus acuminatus* Torr. & Hook., and *Fimbristylis autumnalis* (L.) R. & S. Here and there some individuals of *Thalia dealbata* Roscoe and *Pontederia cordata* L. may be noticed, whereas other parts of the shores are practically bare. Mud flats



UPHOF: PLANTS IN SOUTHEASTERN MISSOURI



UPHOF: PLANTS IN SOUTHEASTERN MISSOURI

along the river during the rainless periods have the same type of vegetation as the mud flats of the lowlands described elsewhere.

The margins of the islands in the Mississippi are also covered by the vegetation above described. On older islands thickets of *Salix nigra* and *Adelia acuminata* are very predominant. *Populus deltoides*, *P. heterophylla*, *Prunus serotina*, and *Betula nigra* are also generally met with. On the largest islands *Taxodium distichum*, *Nyssa sylvatica*, and such trees and shrubs as belong to the true swamp forests often appear.

LITERATURE

1. **Bush, B. F.** Notes on a list of plants collected in southeastern Missouri. Ann. Rept. Mo. Bot. Gard. **5**. 1894.
2. **Daniels, F.** The flora of Columbia, Missouri, and vicinity. Univ. of Mo. Studies I, no. 2.
3. **Hus, H.** An ecological cross section of the Mississippi River in the region of St. Louis, Mo. Ann. Rept. Mo. Bot. Gard. **19**. 1908.
4. **Mackenzie, K. K.** Manual of the flora of Jackson County, Missouri. 1902.

EXPLANATION OF PLATES

PLATE I

Upper left: *Hymenocallis occidentalis* in lowlands; Dunklin County.

Upper right: *Monarda Bradburiana* under young oaks; in hills, western Butler County.

Lower left: *Echinacea purpurea* among several other species of perennial plants; near the edge of a forest, Iron County.

Lower right: Hills covered with *Quercus marilandica;* in front, a specimen of *Nyssa sylvatica*, Butler County.

PLATE II

Upper left: A pure stand of *Nyssa sylvatica;* Butler County.

Upper right: *Pinus echinata;* Carter County.

Lower left: *Taxodium distichum* formation; a mixed forest in the background; eastern Butler County.

Lower right: Mixed forest growth along a stream at the base of a hill; undergrowth largely composed of *Impatiens fulva;* Ripley County.

METHODS OF HEALING IN SOME ALGAL CELLS

Susan P. Nichols

(Received for publication April 30, 1921)

Introduction

At the present time very little is known regarding the method of healing in mature plant cells. The researches of Kite ('13), Chambers ('17), and Seifriz ('18) have been devoted principally to invertebrates and to ova. Their interest was directed to the study of the structure and character of the cell contents as revealed by dissection, rather than to the later reactions to the injury. The repair of the injury and subsequent growth of a few ova have been recorded, but these studies did not involve the rebuilding of plant cell walls. The difficulty of piercing plant cell walls seems to have prevented work along this line.

Kite, in his initial work, dissected Spirogyra and found that

> The cellulose wall is enormously cohesive. It is cut or punctured with extremely fine Jena glass needles with considerable difficulty. The outer surface is covered by an almost invisible soft gel. . . . The protoplasm of plant cells is much more dilute or less rigid than that of animals.

Chambers used the ova of Fucus, which did not involve the study of wall or plastids.

Seifriz, in his studies of plant structure, avoided forms with definite cell walls, with the exception of pollen tubes, the walls of which are extremely delicate. In speaking of oögonia of Fucus, Seifriz states:

> If these oögonia are teased out at a very early age they can be entered by a sharp needle. Very soon, however, the outer wall (exochiton) becomes too hard to be penetrated.

Topler ('03) in some experiments on injured or sectioned cells of *Bornetia secundiflora* found that the protoplasm remaining in a wounded cell would form a new wall. The amount of protoplasm lost in his experiments was sufficient to cause the remaining protoplasm to contract from the old wall against the adjacent uninjured cell of the filament, where it sometimes assumed an abnormal shape. A new wall was then developed by this protoplasm, forming a new cell.

The present article deals with some of the reactions of mature plant cells when punctured.

Material

Both fresh- and salt-water algae were used in the following described experiments. The fresh-water forms were collected near Oberlin, Ohio, and grown in culture in the laboratory. The marine forms were studied in the Biological Laboratory at South Harpswell, Maine. The writer wishes to express her appreciation to the directors, Dr. J. S. Kingsley and Dr. J. L. Conel, for the interest and kindness shown during the summers of 1919 and 1920.

Any form that will live under cultural conditions can be treated by the following methods. This study is confined to Nitella, Chara, and a few of the coarser filamentous algae.

METHODS

The material was mounted in water on a slide and punctured free-hand under a low-power objective. In order to locate a given puncture readily in Nitella and Chara, the distance of the injury from a given node was recorded; in the filamentous forms every alternate cell was punctured. After puncturing, the material was grown in small culture dishes; and careful daily observations were recorded for a month. It was found that punctures made free-hand, with a steel needle ground to a very fine point, gave satisfactory results. This method did not require the time necessary for the more elaborate technique with the Barber pipette holder. In puncturing a large number of cells, punctures of all sizes and depths were obtained. The amount of internal disturbance was estimated by the amount of protoplasm lost and by the movement of the granules in the interior of the cell.

OBSERVATIONS

Vaucheria. The wall of Vaucheria consists of a thin, resistant membrane which is so very pliable that it is difficult to puncture it without injury to the remainder of the filament. If the filament is very turgid, a quick, sharp thrust of the needle will penetrate the wall successfully. The protoplasm is somewhat viscous and the flow is slight. The protruding protoplasm does not diffuse into the water, but forms a globular mass filled with plastids.

In the nine filaments studied the punctured portion was not separated later by a septum from the main thallus, but healed normally.

The delicate character of the plants and the fact that the readjustment of the plastids, as described by Klemm ('94), renders it very difficult to locate the injury after twenty-four or forty-eight hours were the determining reasons for not continuing the study of this form.

Cladophora. Both marine and fresh-water forms of Cladophora were studied. The cell just beneath a branch was selected for a base, and from this base every alternate cell was punctured both in the main axis and in the branch. In the two or three species experimented with, the puncture healed readily, but the later response to the internal disturbance was so unusual that I have reserved this and allied forms for later study.

Chara. In Chara the leaves and cortical cells from the growing point to the mature nodes were punctured. Unless the puncture was very small

(17–34 microns), the escaping protoplasm immediately diffused in the water, rendering it very difficult to estimate the extent of the protoplasmic loss. In a few cases, when the hole was very small (17–34 microns), a delicate film formed over the globule of protruding protoplasm. This globule continued to increase in size, apparently by the absorption of water.

The only movement of chloroplastids loosened by the puncturing occurred within a distance of 34 to 68 microns from the margin of the wound. Even when the hole was very small, rotation of the protoplasm immediately ceased and was not resumed. The original turgidity was also lost and was not regained. This was clearly shown by the invagination of the cross walls from the pressure of the adjacent cells.

Nitella. The internodes of Nitella proved to be unusually favorable material for the study of punctured cells. By puncturing at intervals of five minutes, it is possible to puncture an internode several times without disastrous results. Cells less than one centimeter in length I have punctured six or seven times. Each puncture was followed by a momentary pause in the protoplasmic movement. Even when the puncture was large and deep, with a corresponding loss of protoplasm, the regular rotation was resumed in less than two minutes. After the sixth and seventh punctures the pause was slightly longer than at first. Ultimately the successive drains on the protoplasm result in the cell losing its turgidity; and when that occurs the wall bends in front of the needle, rendering it difficult or impossible to puncture successfully. Although the cell may not be sufficiently turgid to puncture, this condition does not seem to cause any retardation in the healing of the previous wounds. The large size of the internodes and the regular arrangement of the plastids, as well as the moving protoplasm, aid in estimating the effect of the wounds.

Each puncture results in a rapid outflow of the cell contents. The protoplasm does not diffuse in the water, but forms globular or ovoid masses enclosed by a delicate film just outside the opening (Pl. III, fig. 1). The flow is slightly spasmodic—periods of rapid flow interrupted by brief pauses resulting in the formation of a number of separate globules. The plastids and other particles carried through the opening continue to move outward until they are lodged against the enclosing film. At first this film is extremely delicate and can be detected only by careful adjustment of the light or by staining. Gradually the entire mass of globules assumes a brownish tint which brings the films into sharp contrast with the surrounding water. After a few days this protruding mass disintegrates and disappears.

When the cell is punctured, the chloroplastids immediately surrounding the opening are torn from the plasma membrane (fig. 1), and either pass through the opening with the outflowing protoplasm, or are carried along in the rotating protoplasmic stream. The amount of internal disturbance caused by the wound is impossible to determine definitely, but some slight impression of it may be gained by observing the movement of granules.

If the puncture is small, the plastids, loosened from the wall, and the other particles moving in the rotating protoplasm do not show any acceleration until very near the opening. If the puncture is large (250–300 microns), the outward flow is spasmodic, a rapid flow following short pauses until the movement ceases. When the puncture is large enough for such movement, the dislodged chloroplastids and the other particles from every portion of the cell are seen to move toward the opening, indicating a very general internal disturbance.

An accumulation of particles in the hole promptly forms a protoplasmic plug closing the opening. In most cases the forming of this plug is greatly facilitated by an accumulation of the "spiny bodies" so peculiar to Nitella, described by Goeppert and Cohn ('49) and by Overton ('90). These bodies form the foundation of the plugs. If the same cell is punctured a number of times, the number of spherical bodies finally becomes exhausted, and the openings are closed by plastids and starch grains only. The other structures described by Klemm ('94), Küster ('99), and Noll ('99), although occasionally seen, do not appear to form any essential portion of the plug. When the "spiny bodies" are lacking, the plug is formed primarily of plastids and starch grains.

Many young growing internodes (5 mm. or less in length) were punctured. These internodes are less turgid than the older cells, and are filled with a denser mass of protoplasm. In every case healing occurred and growth was resumed so that measurable growth was recorded in twenty-four hours.

Sections made from material killed fifteen minutes after puncturing do not show any indication of the formation of a membrane to cut off the injured protoplasm. The globular bodies forming the plug are crowded at that time into a compact mass closing the opening. The inner surfaces of these bodies are still imbedded in the cytoplasm of the cell, and their spiny projections are unmodified. Before this material was killed rotation had been resumed, and the cytoplasm had been moving normally for 10 to 12 minutes. As the particles in the flowing protoplasm come against the plug they are slightly checked in their movement; and frequently this results in a somewhat denser, thicker mass of protoplasm just within and beyond the opening. The remainder of the cell does not show any modification in its protoplasmic structure.

In sections of material killed one hour after puncturing, it is possible to detect the new membrane forming (fig. 3). This membrane is clearly defined near the cell wall, a short distance back from the opening, but is rather indefinite and indistinct toward the interior of the cell. The membrane does not develop as a uniform, symmetrical invagination, but as a very irregular, convoluted film, which may pass between two adjacent pyrenoids with their associated starch grains, or over one spiny proteid body and outside the next. There is no structural difference visible between the pyrenoids and proteid bodies excluded and those retained by the new membrane. After twenty-four hours the membrane is completely formed over the base of the plug, and a thin film of wall is developed. This new wall can be traced at this time only for a short distance from the opening, where it gradually grows thinner and finally disappears. It thickens very slowly, for after three days it is only slightly more pronounced; though by this time the membrane or, rather, the new wall layers appear to extend farther outward from the wound over the cell. Ultimately the new wall encloses the entire cell, and the healing of the wound is completed as in Chaetomorpha.

Chaetomorpha melagonium f. *typica* (Web. & Mohr) Kützing. This marine Chaetomorpha was also a very satisfactory form for study. The material occurred in great abundance among the fronds of *Chondrus crispus*, and was easy to collect at low tide. The large size of the cells, the character of the cell wall, and the fact that it grows well in culture were factors determining the selection of this species.

The cell walls are tough, elastic, thick, and striated; and their inner surfaces are very finely corrugated. The corrugations usually run in a slightly spiral manner from one end of the cell to the other. They do not appear to be formed by overlapping lamellae, such as West and Hood ('11) found in Trentepohlia, but are rather slight folds of the inner membrane.

The turgid condition of the cells and the elasticity of the walls are essential factors in piercing these tough, pliable walls. When the needle is withdrawn, the contents of the cell slowly flow out and diffuse through the water. There is no surface film formed over the protruding protoplasm, as in the case of Nitella and many other algae; but the protoplasm, together with the chloroplastids, starch grains, and other particles, spreads through the water until it is no longer possible to trace them. Removing the needle causes a slight current in the water, which aids in scattering the particles and also determines the direction of their more rapid dispersal (fig. 2). Finally the starch grains and plastids accumulate at the opening until a plug is formed and all movement ceases. This plug seems to harden, and thus effectually closes the hole; and the cell may be repunctured in forty-five minutes or an hour, according to the size of the hole. The great elasticity of the wall is doubtless a material aid in checking the outflow. The extreme elasticity of these walls is strikingly illustrated if a cell is cut across one end, when the wall frequently contracts until the lumen of the cell is reduced to two thirds or even one half its original diameter.

At the end of two or three hours all the exuded contents have disappeared with the exception of a small, indefinite, discolored mass immediately over the opening; finally, after two or three days, this also disintegrates. The internal plug is still evident, but gradually grows less and less pronounced until it is difficult or impossible to locate the smaller punctures. The larger punctures (200–300 microns) are still visible at the end of a month, although less conspicuous than during the first week.

Cells plasmolyzed forty-five minutes or an hour after puncturing show that a new plasma membrane is growing inward, cutting off a portion of the injured protoplasm (fig. 4, *b*). At the end of an hour the plasma membrane has not completely separated the plug from the protoplasm of the cell, as a slender protoplasmic isthmus still connects the two (fig. 4). Previous to plasmolyzing it is impossible to distinguish any membrane or separation of the protoplasm near the puncture; but in plasmolysis the contracting membrane, drawing the plastids and pyrenoids away from the wall and plug, reveals the region of its formation and the extent of its growth. That a new membrane is formed is shown by the fact that the old membrane is still visible, extending across the space from the new membrane to the puncture (fig. 4, *a*). The space within the old membrane and between the plug and the plasmolyzed protoplasm is filled with a very finely granular substance, similar to cytoplasm in appearance, in which there are occasional coarser granules (fig. 4). At this time it is impossible to determine definitely whether the new membrane lines the entire cell or not, but this seems hardly probable in view of the later developments in the cell. Ultimately the membrane completes its growth, separating the plug from the protoplasm of the cell. As it is possible to repuncture the cell before the membrane has completed its growth, the hardening of the protoplasm to form the plug must effectively close the opening. This ingrowing membrane occasionally will form an extra loop, cutting out a deeply imbedded pyrenoid with its surrounding starch grains and cytoplasm. It is difficult to understand why this should occur, since this excluded pyrenoid does not appear different in any respect from those immediately adjacent but within the membrane (figs. 5, 6).

After the new membrane has formed, the typical lamellose wall begins to appear. But as this wall follows the much convoluted membrane where it is pressed against the plug of pyrenoids and plastids, it is very irregular in outline (figs. 5, 6). It is very difficult to trace the new wall layers back against the old wall. Apparently the first layers gradually grow thinner as they extend back from the injury until they disappear (fig. 6, *a*). The later layers appear to line the entire cell. If the first layers are formed only over the plug and not uniformly over the entire cell, it would seem probable that they are formed by the newer portions of the membrane which is an outgrowth of the original membrane still lining the remainder of the cell. As the new wall increases in thickness it separates the old injured portion of the membrane from the new.

Discussion

The protoplasm in the different species of algae varies greatly in consistency. All degrees of viscosity can be found, from the extremely thin, watery fluid of Chara to the dense, viscous, slightly yellowish mass in the marine Cladophoras. In some species the protoplasm fails to diffuse in the water when the walls are punctured; in others the contents diffuse rapidly. In one case, that of Chara, this diffusion is so rapid and perfect that it is impossible to follow the movement under the microscope; the flow is not checked and the cells die. In all other cases in which the contents diffuse in the water, the flow is checked by the accumulation of particles and plastids in the opening, and healing proceeds normally.

When the protoplasm fails to diffuse in the water the film formed does not seem like a plasma membrane, but more nearly of the nature of a contact film. This is suggested by the fact that this film does not function in any active healing process, although the enclosing protoplasm is in close communication with the protoplasm of the cell. Again a new plasma membrane is formed slowly within the cell by growth from the old membrane. The present experiment has not revealed the exact origin of this membrane; but it seems probable that, in puncturing, a certain radius of the plasma membrane is injured, and that the new membrane begins to form at the outer margin of this region as an outgrowth of the original membrane.

In many cases a cell was torn or cut near one end. In the majority of such cases the major portion of the protoplasm immediately escaped through the open end, resulting in the final death of the cell. In one or two Chaetomorpha cells, a portion of the protoplasm which failed to escape formed an ovoid or globular mass next to the injured cell. This mass of protoplasm was covered by a delicate film, but a wall never developed, as has been described by Topler in his experiments with Bornetia. The mass disintegrated in two or three days.

It is a little difficult to determine Seifriz's exact meaning in his discussion of his only experiment with a cell wall, that of the pollen tube, for when discussing the experiment he says:

> The membrane formed in repairing a tear is of the same character as the original. This is not true in those instances where the wall is of cellulose, as in pollen tubes and plant embryos; for the enclosing surface layer of protoplasm is but a transformed portion of the living substance, the result of an immediate conversion of liquid plasma into a rigid gel of greater molar concentration (Pfeffer, 1891, p. 194).

In this statement he has failed to draw a clear distinction between the cell wall, the plasma membrane, and the film formed over the exuded protoplasm.

From the experiments on the living algal cells which have nonmiscible protoplasm, it would seem that the film formed has no functional value, but later an internal membrane develops which subsequently forms a new cell wall. Certainly my experiments on the algal cell do not substantiate Seifriz's statement that

> Miscibility precludes the presence of a membrane. Normal protoplasm is always capable of membrane formation. Therefore normal protoplasm can at no time be miscible.

The formation of an internal membrane in the algae develops more slowly than the similar structure mentioned by Chambers, of which he writes:

> If a tear of the surface layer or of any part of the cytoplasm be so injurious that disorganization sets in, a film may form around the disorganized area, separating it from the sound cytoplasm. The recovery of an injured cell is only brought about by the formation of a membrane-like film which prevents extension of the injury. A succession of films may form, as one after the other they succumb to the steady advance of the destroying process; and the film which finally holds out may enclose only a fraction of the original cell, but what it encloses will be normal protoplasm.

In the algal cell it is impossible to feel that the new membrane cuts out all the injured contents, since the disturbance is very evidently felt throughout the cell, as shown by the moving particles and also by the fact that the protoplasm immediately within the new membrane appears as dense and disorganized as the portion cut off as a plug. Obviously this membrane cannot serve in checking the loss of protoplasm from the cell, as that movement has ceased some time previous to its formation.

The presence of an enclosing wall is doubtless an important factor in developing various divergences from the responses of an ovum. One of the most essential of these is the readiness with which the loss of protoplasm is checked by plugging the gap in the wall. Then, also, the wall offers an effective support for the new membrane and new wall.

The influence controlling the development and direction of growth of this new membrane may be similar to the stimuli acting in the case of spore formation as described by Harper ('99, '00) for Pilobolus and Fuligo. He found that

> There is no differentiation of hyaline zones or other special regions prior to the formation of the cleavage furrows. Furthermore the nuclei show no special distribution about the cleavage planes. It is quite common to find a group of nuclei on one side of a cleavage furrow, while they are lacking over a considerable area on the opposite side. There is no indication whatever at this stage that the nuclei exert any direct influence on the orientation of the cleavage planes.
>
> If we examine the protoplasm immediately in front of these cleavage furrows also, we find it without differentiation of any sort which would indicate the direction which the furrow will take.

After discussing the influence that the nuclei may exert on the later stages of spore formation, he states:

> On the other hand it is quite possible to assume that cleavage throughout is controlled by the cytoplasm, at first with little reference to the distribution of the nuclei, but later with special reference to the formation of uninucleated cells.

In the separation of the plug, the cytoplasm appears to be the only controlling agent, for there is no visible movement of the nuclei toward the injured region. In the multinucleate forms discussed, a nucleus is found occasionally near the injury; but as nuclei are distributed throughout the cell, some must inevitably be carried out through the opening and lost, while others would be near the puncture when the movement ceases.

Even if several nuclei are lost, a sufficient number remains to maintain the vitality of the cell. In a Spirogyra cell which was punctured the single nucleus was carried through the hole and no recovery followed. Although the hole was small and the protoplasmic loss slight, the cell failed to recover its turgidity.

In Vaucheria the extremely thin membranous walls give so easily under the pressure of the needle that it is very difficult to puncture these walls unless the cell possesses a high degree of turgor. This is also true of Spirogyra, although the wall is much thicker. The tough walls of Spirogyra are the most difficult of any I have attempted. On the other hand, the walls of Chara, Nitella, and Chaetomorpha have a certain amount of rigidity in their structure which greatly aids in puncturing. There does not seem to be any relation between the type of wall and the viscosity of the enclosed protoplasm. Chaetomorpha, with its heavy, striate wall, has a thin, miscible protoplasm, while Cladophora, with an equally heavy wall, has very dense, nonmiscible contents.

It has not proved possible to estimate the total amount of protoplasm which may be lost by the injured cells. That this loss may be very large in some cases is evidenced by the fact that Nitella cells can be punctured six or seven times in an hour and still recover. There are no visible ill effects from the loss of protoplasm, and future growth appears normal in every respect. If the culture becomes infected with either bacteria or molds, the punctured cells do not seem any more susceptible than the uninjured. Yet, if the plants are placed in an unfavorable environment, the injured cells are always the first to succumb, indicating that there has been a lowering of their vitality.

I wish to thank Professor R. A. Harper and Professor F. O. Grover for their interest and encouragement during the progress of this study.

Summary

1. All but one of the algae studied are able to heal a wound.

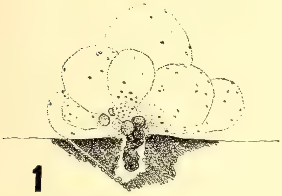
2. The density of the protoplasm varies from a very liquid condition in some species to a quite viscous condition in other species.

3. No correlation was discovered between the density of the protoplasm and the character of the cell wall.

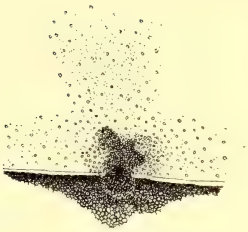
4. The exuded protoplasm may or may not be miscible with water.

5. If the exuded protoplasm is nonmiscible, the film formed over the escaping protoplasm is not comparable with the plasma membrane.

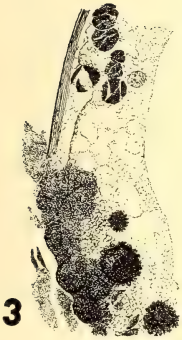
6. The puncture is not closed by a film or membrane but by an accumulation of plastids, pyrenoids, and starch granules in the opening.



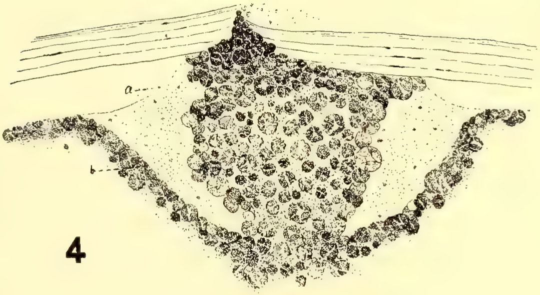
1



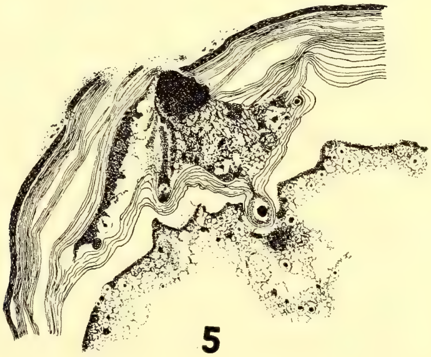
2



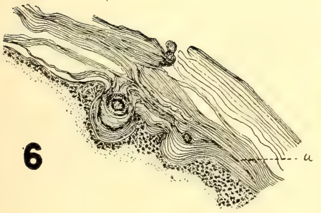
3



4



5



6

NICHOLS: HEALING IN ALGAL CELLS

7. A new plasma membrane grows inward from the old membrane and separates a portion of the protoplasm filling the puncture from the remainder of the cell.

8. A new wall is gradually formed by this membrane, and the healing is complete.

OBERLIN COLLEGE,
OBERLIN, OHIO

LITERATURE CITED

Chambers, R. ('17). Microdissection studies I. The visible structure of the cell protoplasm and death changes. Amer. Jour. Physiol. **34**: 1–2. 1917.

Goeppert, H. R., and Cohn, F. ('49). Ueber die Rotation des Zellinhalts in *Nitella flexilis.* Bot. Zeit. **7**: 665–673, 681–691, 697–705, 713–719. 1849.

Kite, G. L. ('13). Studies on the physical properties of protoplasm I. The physical properties of certain animal and plant cells. Amer. Jour. Physiol. **32**: 146–164. 1913.

Klemm, P. ('94). Ueber die Regenerationsvorgänge bei den Siphonaceen. Flora **78**: 19–41. Taf. V, VI. 1894.

Küster, E. ('99). Ueber Derbesia und Bryopsis. Ber. Deutsch. Bot. Ges. **17**: 77–83. Taf. VI. 1899.

Harper, R. A. ('99). Cell-division in sporangia and asci. Annals of Bot. **13**: 467–523. Pls. XXIV–XXVI. 1899.

——— ('00). Cell and nuclear division in *Fuligo varians.* Bot. Gaz. **30**: 217–249. Pl. XIV. 1900.

Noll, F. ('99). Die geformte Protein im Zellsafte von Derbesia. Ber. Deutsch. Bot. Ges. **17**: 302–306. 1899.

Overton, E. ('90). Beiträge zur Histologie und Physiologie der Characeen. Bot. Centralbl. **44**: 1–10. 1890.

Seifriz, W. ('18). Observations on the structure of protoplasm by aid of microdissection. Biol. Bull. **34**: 307–324. 1918.

Topler, F. ('03). Ueber Vernarbung und Wundreiz in Algenzellen. Ber. Deutsch. Bot. Ges. **21**: 291–300. Taf. XIV. 1903.

West, G. S., and Hood, O. E. ('11). The structure of the cell wall and the apical growth in the genus Trentepohlia. New Phyt. **10**: 241–249. 1911.

EXPLANATION OF PLATE III

FIG. 1. Nitella with escaping nonmiscible protoplasm. × 160.

FIG. 2. Chaetomorpha with escaping miscible protoplasm. × 160.

FIG. 3. Nitella: section showing new membrane forming. × 160.

FIG. 4. Chaetomorpha plasmolyzed to show the new (*b*) and old (*a*) membranes. × 160.

FIG. 5. Chaetomorpha: section showing a new wall formed over the plug fifteen days after puncturing. × 160.

FIG. 6. Chaetomorpha: section showing a new wall well formed three weeks after puncturing. × 160.

THE PRESENT STATUS OF SCOLOPENDRIUM IN NEW YORK STATE

Mabel R. Hunter

(Received for publication May 6, 1921)

The purpose of this paper is to offer data concerning the occurrence and abundance of *Scolopendrium vulgare* Sm. in the central New York area. Its former distribution is also considered and comparisons are made in an effort to throw some light upon its probable future. The work was begun in the autumn of 1916, and was completed in January, 1921. Data concerning several of the colonies have been obtained from field notes and other information kindly placed at my disposal by Dr. Loren C. Petry of Syracuse University, Dr. M. S. Markle of Earlham College, and Dr. John B. Todd, a physician of Syracuse.

While certain topographic conditions due to glacial action are coincident with the occurrence of Scolopendrium in this region, it is not deemed necessary to give details of this topography[1] beyond a brief mention of the three well-defined situations under which Scolopendrium occurs. These situations are: (*a*) on the south slopes of glacial channels, which usually extend almost due east and west; (*b*) on the south and west slopes of deep plunge basins which head such channels; (*c*) on the west slopes of steep gorges cut by post-glacial streams flowing in a northerly direction. The latter situation occurs only at the Chittenango and Perryville Falls stations. These three situations are all similar in certain respects. The slopes are usually bordered by an overhanging cliff, and in all instances where this is the case Scolopendrium occurs well below the cliff, about midway of the slope. A further characteristic of its occurrence is that of growing in distinct groups or colonies, with no scattered plants between.

In this paper the term "station" is applied to the widely separated regions; distinct areas within the stations are termed "substations," and these may in turn be divided into "colonies."

The first comprehensive account of the occurrence of Scolopendrium in the New York area was published by Maxon (2) in 1900. In this paper he gives an account of the discovery of the various stations of Scolopendrium in America, along with facts concerning its condition at various stations at that time. Since 1916 the stations in central New York cited by Maxon have been relocated, the surrounding areas examined, and all discoveries carefully mapped (figs. 1, 2). More detailed maps are on file in the Department of Botany, Syracuse University, and copies of these are available to anyone interested. For convenience the stations will be discussed in the order in which Maxon describes them.

---

[1] For a discussion of the physiography of this area the reader is referred to the series of papers on this subject by Dr. H. L. Fairchild, and especially to his *Glacial Waters in Central New York*, N. Y. State Museum Bull. 127. Albany, 1909.

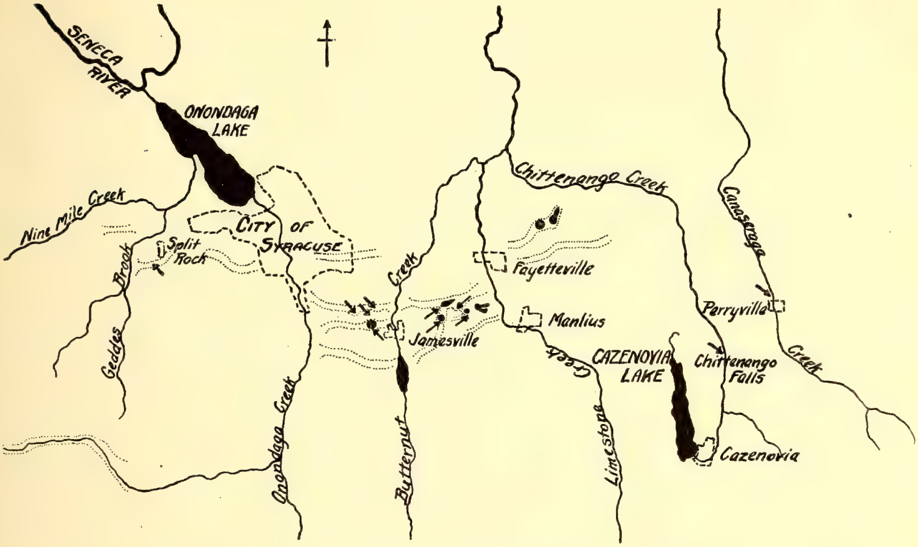

Seneca River
Onondaga Lake
Nine Mile Creek
City of Syracuse
Split Rock
Brook
Geddes
Onondaga Creek
Butternut Creek
Jamesville
Fayetteville
Manlius
Chittenango Creek
Creek
Cazenovia Lake
Limestone
Perryville
Chittenango Falls
Canaseraga Creek
Cazenovia

FIG. 1. Map showing Central New York stations for Scolopendrium. Stations are indicated by arrows; cliffs bordering glacial channels and plunge basins are shown by dotted lines. Scale: 1 inch = 8 miles.

I. GEDDES (SPLIT ROCK) STATION

Scolopendrium was first discovered in America by Frederick Pursh (7) in 1807 on "Geddes plantation," west of Syracuse and near the present village of Split Rock. In 1866 Mr. J. A. Paine (3) searched the region but failed to find Pursh's station. In 1879 the Syracuse Botanical Club (8) rediscovered Scolopendrium at what seemed to be Pursh's station. Reports show that it flourished there until 1895 when it was thought to have been exterminated by quarrying operations of the Solvay Process Company.

There is some doubt as to the exact location of Pursh's original station. In his *Flora Americae Septentrionalis* (6) Pursh mentions "shady woods among loose rocks." In his *Journal* (7) he refers to

> A deep valley where we ascended a steep rocky hill; here large masses of rock seemed to be piled up or turned over on one another in such a confused manner that it has left chasms between them which sometimes appear like caves.

It is quite probable that the deep valley referred to by Pursh is the one through which the Syracuse and Auburn Electric Railway now runs. The quarrying operations referred to were confined to the region south of this valley, and resulted in the removal of a mass of rock of probably fifty feet in thickness all along the valley. A search of this valley, known as Split Rock Ravine, by Dr. Petry in the autumn of 1918 resulted in the discovery of a single small colony. This colony, consisting of six mature[1] plants, six young plants, and three groups of prothallia, is located on the south slope about fifty feet down from the base of the low cliff. It is just west of a dump of the Solvay picric acid plant and midway between it and a rock slide. It has since been learned that this colony had been discovered several years earlier by Dr. John B. Todd.

In view of the extended lapse of time between the various discoveries of Scolopendrium at Split Rock, together with the lack of precise information as to the location of the original colony described by Pursh, it is difficult to decide whether the three reports (Pursh, 1807; Syracuse Botanical Club, 1879; Petry, 1918) refer to the same or to different colonies. The quarrying operations have disturbed the region to such an extent that it is impossible to trace the details of topography mentioned in the former description, but the traces of the original general topography indicate that the present colony is at least in the vicinity of Pursh's original colony. The fact that observations elsewhere offer no evidence of formation of new colonies leads to the conclusion that the present small colony in Split Rock Ravine is either a remnant or an outlier of Pursh's original station.

II. Chittenango Falls Station

At this station Chittenango Creek flows in a northerly direction and plunges over a precipice one hundred feet or more in height, forming a deep gorge below. The west bank of this gorge is exceedingly steep and rocky, while the east bank is sloping and covered with soil. A short distance below the fall two ravines enter the gorge from the west, at right angles to the stream. The discovery of Scolopendrium at this station by William Cooper in 1830 was first published by Gray (1) in 1866. Maxon (2) reported in 1900 that it grew scattered along the west bank below the falls for "a distance of nearly a quarter of a mile." At the present time Scolopendrium occurs abundantly on the south bank of the second ravine below the gorge and from that point along the west bank of the main gorge up to within about 150 yards of the falls. Here, as elsewhere, it shows the characteristic habit of growing in groups or colonies.

---

[1] In dealing with the actual number of plants it has been found convenient to classify them into three groups: (*a*) mature plants, those having at least one leaf bearing sori; (*b*) young plants, those having one or more leaves of typical shape but not bearing sori; (*c*) sporelings and prothallia. This last group includes all the stages from the time the prothallia are recognizable up to the time when the leaves take on the characteristic shape.

The prothallia and sporelings almost invariably occur in groups which may contain several dozen individuals; these groups seldom contain prothallia of other species, but when such mixed groups do occur, the Scolopendrium prothallia are easily distinguished from the other species. In tabulations, prothallia and sporelings are listed by groups, and no attempt has been made to count the individuals.

III. Perryville Falls Station

The Perryville Falls station lies about three miles northeast of the Chittenango Falls station, at the point where Canaseraga Creek has formed a deep gorge. Scolopendrium was discovered here in 1898 by Miss Murray Ledyard of Cazenovia (4).

In August, 1919, this station was visited and the entire region below the falls in which Scolopendrium might be expected to occur was explored. At a distance estimated to be 150 yards down stream from the crest of the falls and 40 feet above the stream level, a colony of thirteen mature plants, three young plants, and two patches of prothallia was located. A little to the south of this was a single young plant, and still nearer the stream bed one mature plant and a group of prothallia were found. Dr. Petry's field notes describing the colony at this time say:

> The plants were vigorous and were fruiting strongly; the sporangia, however, were not nearly mature at this time. The plants here were considerably smaller than those at the Chittenango Falls station. There were, nevertheless, the normal number of leaves. Altogether it may be said there is no evidence that the colony is not maintaining itself.

A second visit in October, 1920, showed some surprising facts. The colonies were again located and the plants counted. Fifteen mature plants, ten young plants, and nineteen groups of prothallia were found. The young, straggling plants had become mature plants, and the groups of prothallia were in a very flourishing condition.

It is to be noted that in the cases of both the Chittenango and the Perryville Falls stations we have the same physiographic conditions, *i.e.*, gorges below waterfalls. In each case the gorge is deep with vertical cliffs and steep slopes covered with *débris*, soil, and broken fragments of the thick-bedded, chert-bearing layers of the Onondaga limestone. Scolopendrium occurs on the west slope of the gorge in each instance, the east slope being of an entirely different botanical make-up.

IV. Jamesville Station

This is at once the best known and most accessible of the New York stations. It consists of a series of colonies scattered through the maze of glacial channels and plunge basins lying on either side of the village of Jamesville. The colony nearest to Syracuse lies not more than three miles outside the city limits, and two colonies (*B*, fig. 2) lie within the Clarke State Reservation. The colonies east from Jamesville lie in very rough country, but all are within easy walking distance of trolley lines. In spite of this, their exact location is known to relatively few people.

A. Howlett's Gorge (*Hanging Valley*) *Substation.* The first discovery of Scolopendrium in the Jamesville region was made by Mr. Lewis Foote (1) in 1866, at Howlett's Gorge, now better known as Hanging Valley. An examination of this area in November, 1920, showed a thrifty colony of somewhat scattered plants on the north side of the ravine. In this particular, namely, its location on a south-facing slope, this colony is unique in the New York area; but equally peculiar is its occurrence on outcrops of Fiddler's Green limestone of the Camillus series, a full hundred feet below the horizon of the Onondaga limestone on which all the other colonies of this area are located.

B. Little Lake (*Green Lake*) *Substation.* In September, 1866, Paine (3) discovered Scolopendrium on the southern slope of the cliff surrounding Little Lake, or Green Lake, as it is now known. Maxon (2) states that

> The plant was formerly very abundant but scarcely half a dozen plants may now be found, owing to the greed of picnickers.

A careful search of this region in November, 1920, disclosed a colony of forty or more thrifty plants high up on the slope south of the lake. To the southeast of the lake, on the west slope of a plunge basin which contains water for a short time each spring, is another colony of fifty or more plants. Both these colonies are within the Clarke State Reservation.

Maxon also mentions the occurrence of Scolopendrium in two wooded ravines from thirty to forty rods north of the lake. This region will be discussed in detail later as the Jamesville Woods substation. It is sufficient to say here that it is at the present time the most promising substation of the Jamesville area.

C. Green Pond Substation. Mr. Paine in the same year found Scolopendrium at Green Pond, a lake similar to Green Lake and located one and one half miles east of Jamesville (*C*, fig. 2). Of this location, Maxon (2) reported that the fern grows

> Pretty well up on the sides at the base of the U cliff which surrounds Green Pond. . . . The plants from the cleared eastern portion become of small size and winter kill badly.

At the present time, Scolopendrium is not found at the eastern side of the lake; its disappearance from this area is probably due to clearing followed by fire. Beginning almost directly south of the center of the lake, the fern occurs very abundantly on the upper part of the slopes along the southern and western sides of the lake. In one area about twenty-five feet square, ninety plants were counted. Along a part of the cliff top, plants in considerable numbers occur on the level ground immediately back of the rim; and in the bottom of a shallow plunge basin a few hundred yards south of the rim a few small but mature plants were found.

D. Rock Gorge Substation. This substation was first discovered by Maxon in 1900, at which time he reported "125 extremely fine plants growing very thriftily." This colony is still in a flourishing condition. However, the small colony reported as located "in a small depression some 40 rods to the westward and back from the amphitheater" has not been rediscovered, although the region has been examined carefully.

E. West White Lake Substation. Southwest of White Lake, and equidistant from it and Green Pond, lies a large plunge basin in which Scolopendrium is very abundant. This colony was not mentioned by Maxon but has been reported by Petry (5). Unfortunately this substation lies directly in the path of development of nearby quarrying operations and will probably be destroyed in the near future.

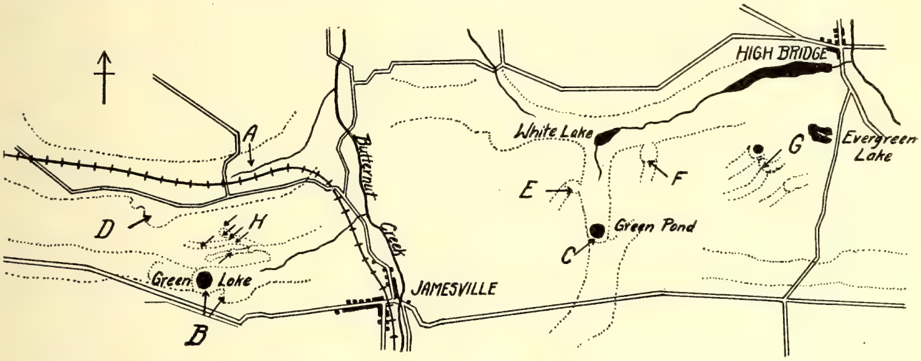


FIG. 2. Detail map of the Jamesville station. Colonies of Scolopendrium are indicated by arrows; cliffs bordering glacial channels and plunge basins are shown by dotted lines. Substations are as follows: *A*, Howlett's Gorge; *B*, Green Lake; *C*, Green Pond; *D*, Rock Gorge; *E*, West White Lake; *F*, East White Lake; *G*, Evergreen Lake; *H*, Jamesville Woods. Scale: 1 inch = 1½ miles.

During the progress of the field studies it has become evident that Scolopendrium is restricted in the central New York area to a very limited habitat, and that it need not be looked for except in regions of a definite topographical character. Systematic search of practically all localities of this character between Split Rock on the west and Perryville Falls on the east has been made, and has resulted in the discovery of two substations not heretofore recorded. It cannot of course be stated positively that Scolopendrium does not occur elsewhere in this area; but the writer believes that no large colonies have been overlooked in the survey.

F. East White Lake Substation. This substation was located some years ago by Dr. Todd, who kindly furnished information with regard to its location. It is located on the south and west slopes of a large plunge basin (*F*, fig. 2), and contains two colonies of approximately twenty and sixty plants respectively.

G. Evergreen Lake Substation. About one half mile to the west of Evergreen Lake (known locally as "Bullhead Pond") lies an extensive area of ravines and plunge basins. A striking feature of the region is a very deep crescent-shaped ravine. On the west slope of this ravine is a thrifty colony of fifty or more plants (*G*, fig. 2). About 200 yards east of this colony, on the same slope, is a single large mature plant. This substation was first discovered in October, 1920.

The data given above answer one of the questions most often asked with regard to Scolopendrium in New York, namely, whether the species has disappeared from any of the recorded stations. Another question of equal interest is whether the number of individuals in the various colonies is dwindling at the present time and thus forecasting the disappearance of the species in this area. To determine this latter point a detailed study of one particular substation, that in Jamesville Woods (*H*, fig. 2), has been made. This locality was selected for study because it is the most accessible area containing more than one colony.

H. The Jamesville Woods Substation. The special study of this substation was begun in the autumn of 1916. The entire area is dissected by a maze of ravines and plunge basins in which the colonies of Scolopendrium occur. A sketch map of the region was made, and the locations of the six colonies found were indicated on this. Each colony was then examined in detail, and a careful census of the plants was made. To accomplish this, each area was divided into strips from ten to twenty feet in width and extending from the bottom to the top of the slope. These strips were then examined in order, beginning each time at the foot of the slope; the plants found were recorded as mature plants, young plants, or groups of prothallia, as already described. The census of selected strips was checked by recounts, and in one instance an entire colony was recounted.

In the autumn of 1920, a second census of the colonies was made by the use of the same methods. The results of the two counts are given in table 1.

TABLE 1. *Jamesville Woods Substation*

Colony	Number of Mature Plants		Number of Young Plants		Number of Groups of Prothallia and Sporelings	
	1916	1920	1916	1920	1916	1920
I	159	197	56	85	38	60
II	109	115	45	36	17	23
III	17	8	4	5	7	3
IV	119	206	35	89	17	52
V	29	20	9	4	6	2
VI	136	92	86	72	35	48
Totals	569	638	235	291	120	188
Increase		69		56		68
Increase percent		12.1		23.8		56.6

As shown by the table, the number of mature plants in the substation increased 12.1 percent in four years. The increase in young plants and groups of prothallia has been even more rapid, and is interpreted to mean that the increase in number of mature plants may be expected to continue in the immediate future.

This increase, however, is not common to all colonies of the substation, colonies III, V, and VI showing a decrease instead. Colonies III and V lie in the same ravine with colony IV, and are in fact extensions of that colony. Colony III occurs on the south slope of a shallow portion of the ravine. The plants grow near the top of the slope, much higher than is usual, and the shallowness of the ravine leaves them considerably exposed. Field notes taken during the 1916 census say, with regard to this colony, that "while the plants here seem to be vigorous they are smaller, less abundant in a given area, and much more exposed; the habitat more nearly resembles . . . the upland forest." The decrease in number of plants revealed by the second census may be taken to indicate that this small colony has moved somewhat too far out of the habitat most suitable for the species.

Colony V is located on nearly level ground at the opposite end of the ravine containing colony IV. This unusual habitat for Scolopendrium has already been mentioned as occurring also in part of the Green Pond substation, but has been found nowhere else. As at colony III, the plants are smaller and more widely scattered. Here also the decrease in numbers is explained by the exposed habitat.

Colony VI, which also showed a decrease in numbers of plants, presents a different situation. This colony is located on the south slope of a deep ravine, under conditions thoroughly typical for Scolopendrium. At the time of the first census it was noted that many of the plants were very superficially rooted; field notes say that "large plants grow on rocks covered with mosses and liverworts." At the time of the second census a condition of drought prevailed in this ravine and these rock coverings of humus were completely desiccated. As a consequence, only those plants which were rooted in crevices between rocks and in other moister situations had survived. It is to be noted that the prothallia which usually occur in these more sheltered situations have increased in this colony at the same time that young and mature plants have decreased in number.

The second census also brought out the fact that not only may colonies change rapidly in number of individuals, but an increase in one portion of a colony may be accompanied by a decrease in another area of the same colony. For example, the notes of the 1916 count of colony IV show that at that time the larger number of plants occurred in the eastern portion of the ravine. In 1920 the western portion of the colony contained by far the greater number of plants. It is hoped to learn more about this shifting of populations within colonies and its relation to the fluctuations in total number of individuals by annual counts of the colonies of the Jamesville Woods substation.

The results of the two counts already completed make it certain that at the present time the number of plants of Scolopendrium in the Jamesville Woods area is increasing. As stated above, a similar conclusion is indicated for the Perryville Falls station by comparison of the conditions there in 1919 and in 1920. These two sets of exact data, considered in connection with the persistence of the species in all its described stations and including its virtual reëstablishment at the Green Lake substation, lead to the conclusion that Scolopendrium is at the present time becoming more abundant.

Summary

1. All described stations for *Scolopendrium vulgare* Sm. in the central New York area have been located, and the fern has been found in greater or less abundance at each.

2. Two new substations for the species have been discovered in the Jamesville area.

3. The number of individual plants in the six colonies of the Jamesville Woods substation was determined in 1916 and again in 1920. Comparison of the data shows that Scolopendrium is becoming more abundant.

Syracuse University,
Syracuse, New York

LITERATURE CITED

1. **Gray, A.** *Scolopendrium officinarum.* Amer. Jour. Arts and Sci. II, **41**: 417. 1866.
2. **Maxon, W. R.** On the occurrence of the hart's-tongue in America. Fernwort Papers 30–46. 1900.
3. **Paine, J. A., Jr.** *Scolopendrium officinarum* in western New York. Amer. Jour. Arts and Sci. II, **42**: 281–283. 1866.
4. **Parsons, Frances T.** A new station for the hart's-tongue fern. Fern Bull. **6**: 74. 1898.
5. **Petry, L. C.** The vegetation of a glacial plunge basin and its relation to temperature. Bull. Torrey Bot. Club **45**: 203–210. 3 figs. 1918.
6. **Pursh, F.** Flora Americae Septentrionalis. London, 1814.
7. ——. A journal of a botanical excursion in the northern parts of the states of Pennsylvania and New York during the year 1807. Edited by T. P. James and issued in 1869. (This reference has not been available to the writer and the quotation is copied from Maxon.)
8. **Rust, Mary O.** Pursh's station for Scolopendrium rediscovered by the Syracuse Botanical Club. Bull. Torrey Bot. Club **6**: 345, 346. 1879.

BIOPHYSICS AS A POINT OF VIEW IN PLANT PHYSIOLOGY[1]

HOWARD E. PULLING

(Received for publication May 14, 1921)

This year is the 300th anniversary of the appearance of Francis Bacon's "Novum Organum, or true suggestions for the interpretation of Nature." It is appropriate and perhaps not presumptuous to take as a text one of Bacon's remarks and as a subject a point of view with respect to our science, for Bacon's work was essentially the presentation of a point of view: "Francis of Verulam thought thus," he said. This text and this subject are contained in his 36th Aphorism:

> We have but one simple method of delivering our sentiments, namely, we must bring men to particulars and their regular series and order.

In other words, it is proposed that we consider the applicability of a quantitative, physical method to plant physiology: the regularity—the mathematical regularity—of the series and order of its "particulars." This applicability, indeed, is implied in the original meaning of the Greek word for physics: a knowledge of the regular successions and relations of events, whether these events be mechanical or vital. It is the Greek equivalent for the Latin word for nature, so that biophysics is but the recharting of a field of knowledge, the traverse survey of which was made by the ancients.

Whatever one may feel regarding the utility of a purely physical point of view in plant physiology, its chief value lies in its offering *another* gateway into the unknown while closing none now existing, for the most securely based hope of progress in plant physiology rests upon the varied attitude of its votaries toward their science, a science that is unable to demand a particular viewpoint from the very nature of its subject matter. Like Bacon, I seek not to discredit the constituted authorities, or their methods, or their desires. But I do propose to call attention to certain possibilities that are not presented in the diet of reading that is regularly offered to those who are completing their apprenticeship as plant physiologists.

The physical edifice is three-storied, at least in practice. The first story, on which the others rest, is the conspicuous one to the outsider. It is the busy, experimental floor that is presided over by *Precision* and is so commonly pictured in the textbooks.

In the second story we find the models that serve to identify all physical processes regardless of where they may be encountered. With them is arranged the collection of laws, theories, and hypotheses that is constantly being augmented by additions from the laboratories below, and that is constantly being altered by the substitution of new items of improved design for those fabricated in the past. It is a temptation to linger here, for there are so many things reminiscent of phenomena in plant physiology. On this floor abstract reasoning is the principal activity. Details of the results achieved below-stairs are sorted, criticized, and classified. Those that are unessential are filed, and the others are built into more and more elaborate models as the skill on the part of the laboratory operatives increases and new and better machinery and tools are acquired.

---

[1] Invitation paper read before the Physiological Section of the Botanical Society of America, in the symposium on biophysics, at Chicago, December 28, 1920.

But it is to the third story that the guide conducts us today. Here are quiet rooms, thick with silence, in which the faiths of a science are kept. These are stored as sketches prepared in the idle moments of genius: skeleton diagrams of the possible relations between many apparently dissimilar categories of data. Some of these diagrams may now be viewed as models on the second floor. Others have been shown to have inherent fallacies, while others are so grand in conception that an infinity of time will be required to set in final place all the details of which they hint.

The largest of these is labeled "Conceptions of the material system." So large is it that one is likely to miss the whole in contemplation of its parts, for the sketch is a patchwork of many, and the light shed by published comments is flickering, so that now one, now another part is illuminated.

It is now purposed to render one aspect of the material system, thus pictured, into concise wording and to contemplate its application to the study of the living plant.

Broadly speaking, the large, composite sketch says: "A system as a whole possesses interrelated properties that may be quantitatively investigated as such without regarding the molecular constitution of the system or the molecular kinetics of its processes, and a quantitative statement of the *laws* of a system as a rule is a helpful antecedent to all *theories* of the cause of its unique behavior." Now the recognition of the living plant as such a system is generally being only unconsciously made in plant physiology. In physical chemistry a system is sometimes defined as an aggregation of matter in, or tending towards, equilibrium. This definition is incomplete, for it makes no mention of the fact that systems possess peculiar properties that characterize the system as a whole, distinguish it from other systems, and can not be obtained by adding together the properties of its components. In general every system has certain properties characteristic only of itself and not deducible from the properties of its parts. This is true of all systems: chemical, mechanical, vital.

Let us now return to the first floor and note what Physics does. She takes systems as she finds them in nature and tries to discover a measurable feature that will serve to identify a class of systems, adjudged a class from their common behavior in some respect. She further seeks to find a number that will, by its magnitude only, indicate the degree to which an individual system of the class is capable of manifesting the activity that identifies the class. She does this without anticipating any theory to account for the activity and without considering that, with respect to other activities, these individual systems of the class may be wholly unrelated.

It is sometimes doubted that this is done in physics. Physics is reputed to be in constant search for explanations of phenomena. That, of course, is true, and no science can abandon attempts along this line. But it is likewise true that physics does not make the search for explanation as the first step. The first step is the formulation of a quantitative law that represents the relation between the activity characteristic of the class and the quantity that symbolically represents the individual system, in other words, a constant whose magnitude may vary in any way whatever from system to system of the same class. When this law is established, the task is undertaken of discovering the law that connects the magnitude of the constant with some measurable feature of the individual system, some characteristic that can be measured when the system is not manifesting the activity under consideration or which for some other reason renders calculation of the constant easier.

Somewhere, at some time during this undertaking, an observation is made that leads an experimenter to connect the behavior of systems in one class with that of systems in another. Gradually thus relationships are perceived, the statements of which are, in fact, the so-called explanations, each law being seen to be a special case of another and "explained" by the more general. I do not mean that each individual physicist progresses in this way, but that, on the whole, this faith that careful measurement of the interrelations of activities of individual systems, the expression of the quantitative relations between the attributes of these systems, always with the view of defining the particular systems each by the magnitude of a measurable dimension, a static character, etc., or by a relation between such characters, a constant in short—faith in this method has been the keystone of progress in physics.

Because this may appear to be but the opinion of an outsider, perhaps it would be best to quote a physicist. Clerk Maxwell's classification of the physical sciences may be summarized thus:

The chief divisions of physics are two:

A. Fundamental science of dynamics, or the doctrine of the motions of bodies as affected by force.

B. Secondary physical sciences. Each has two divisions or stages. To quote:[2]

> In the elementary stage it is occupied in deducing from the observed phenomena certain general laws and then employing these laws in the calculation of all varieties of phenomena. In the dynamical stage the general laws already discovered are analyzed and shown to be equivalent to certain forms of the dynamical relations of a connected system and the attempt

---

[2] Maxwell, J. Clerk. Physical sciences. Encyc. Brit. **19**: 1–3. The R. S. Peale reprint of the 9th edition. Chicago, 1892.

> is made to discover the nature of the dynamical systems of which the observed phenomena are the motions. The dynamical theories of the different physical sciences are in very different stages of development and in almost all of them a sound knowledge of the subject is best acquired by adopting, at least at first, the method we have called "elementary," that is to say, the study of the connection of the phenomena *peculiar to the science* without reference to *any* dynamical explanations or hypotheses.

The italics are mine.

A glance over some of these secondary sciences (which include such theoretically unrelated topics as *elasticity of figure in solids*, *viscosity*, *cohesion of liquids*, *thermodynamics*, *geometrical optics*, *electricity and magnetism*) and a perusal of a standard text on the subject must be convincing that physics does take systems as she finds them in nature and finds mathematical relationships between their conveniently measured attributes, without considering at the outset any dynamical theory to account for the properties. And, lastly, it must be noted that in the mathematical expression, the concrete, individual system is represented by one or more constants whose magnitudes are determined by independent measurements on the system itself.

It has been objected that plants are far too complicated systems to have any such constants, that the variation of one plant from another of the same species would be so great that there would be no value in the constant, that the evaluation of the constant would involve its determination for each plant and would require so much work as to defeat the object. Perhaps it is useless to discuss the matter in advance, but there are many reasons for doubting the cogency of these arguments. Indeed, the more one ponders the matter the more one is convinced that estimating the degree of potential activity of systems by inspections of their surface features is a universal method of the human race. In the affairs of life we say that one who is successful in the exercise of this method has judgment; symptomology in medicine is the systematization of the results of such an attitude.

The botanists of yesterday recorded numberless instances of morphological characters that are always found associated—or correlated, as they said. It is, of course, recognized that structure and function have some sort of interrelation, and a good deal of work has been directed toward finding the so-called causes of the interrelation. It is becoming likewise probable that a great number, at least, of physiological processes are correlated. All this would be predictable from the point of view of the physical system, predictable, that is, in a rough, general sense; for, of course, it has required much scientific imagination to discover these correlations and it will involve a great deal of tedious work to evaluate the degree of the interrelation. Now, may not the relative magnitudes of morphological features be a reflection of, or be quantitatively associated with, the plant's ability to carry on its life processes, *i.e.*, the physiological processes that we find such difficulty in measuring? If morphological characters have physiological bases, may not morphological correlation be associated with physiological correlation?

In this we come to the root of the matter. The attention of plant physiologists has ostensibly been directed chiefly to the relations between plant and environment. I say ostensibly because, although the environment is measured as such, the plant is not. The plant is always measured in terms of the results of the interaction between environment and plant. We have no word for the plant itself, its inherited potentialities, the features that make it an individual. We know that plants of the same species, variety, and crop are physiologically different and remain so all their lives. These quantitative differences we vaguely ascribe to "individual variability," and on it we blame many, if not most, of our experimental difficulties.

To present the matter from another standpoint and thus, perhaps, make it clearer: suppose we could measure this internal, physiological constitution of the plant, that we knew all there was to know about a plant's *ability* to operate. Suppose we allowed these plants to grow in hermetically sealed boxes, of various sizes, shapes, and contents, the contents being wholly unknown. Suppose that after an interval of time we should analyze the contents of the boxes by every means at our command. How much knowledge would be acquired regarding the plant's relations with its environment? How much of the content of the box was put there by the plant and how much was residue? What compounds were formed by the plant and what by interaction of plant excretion and box content? How many repetitions of the experiment would be necessary before sure knowledge would be gained? True, we might select the boxes as to uniformity of size, shape, color, etc., but what would that avail if there were no relation between externals and internals? Now, restate this, replacing the words box and box-content by plant, and *vice versa*, and we have a picture of the present situation.

It must be evident that the saving feature in the case of the measurable environment and the unknown plant, the situation in which we actually find ourselves, is that there *is* a relation between externals and internals, and that a plant's activities do not have an indefinitely large range but are limited. Why then do we have so much difficulty in ascertaining the relation between environment and plant? In the absence of a problem's solution no one, of course, can state all reasons for failure to find one, but there are considerations that offer probable answers to the question. In the first place, the environment has not been completely stated in any instance, nor consciously duplicated in any two instances. Accordingly, the situation is more complicated than the term "measurable environment" might seem to indicate. As has so many times been pointed out, it should be measured and controlled—and if I may interject a remark, a first-hand acquaintance with the first story of the physics building is required for this: a precise workable equipment of laboratory knowledge.

In the second place, since we have little knowledge of the relations within the plant, of the interrelations of the plant's activities, even in an environment whose composition is measured, but which in kind and quantity of components is chosen at random, there is little chance of success because the main current or trend of the plant's activity may, and surely does, change if the environment is sufficiently altered, or altered in particular respects. We know enough on this point to be sure that such an altered activity is common. In the third place, since we select our plants on the basis of the magnitude of an external character such as height of seedling at a certain age, weight of seed, color of seed, etc., and not on the basis of a *relation* between external characters, we are again dealing, not with the plant's internal constitution but with the product of the interactivity of environment and plant, even though that interactivity occurred before the materials came to hand, that is, during seed formation. This should be taken to indicate, not that work of this kind is without value, but that it would progress more rapidly and more surely if the ever-present variable of plant constitution could be evaluated.

This brings us to another objection. Suppose for the sake of argument, it may be said, that internal activity and external character are related quantitatively, does it follow that the relationship is either direct or easy of discernment? Of course not; if the relationship were direct and easy it probably would have been noticed long since. Hope lies in the general success of such endeavors in physics and in physical chemistry, but this point of view is not proposed as a panacea for all experimental difficulties; it is a proposal for a campaign rather than for a *coup*. A little later I shall attempt to consider an avenue of approach, general considerations upon which methods may be based, and this will probably prove a more satisfactory form of answer.

It has been objected that, even supposing we have found a mathematical expression connecting the activities of a small number of individual plants with their measurable characters, such a relation must involve one or more constants peculiar to the individual, and since these constants will vary in magnitude from individual to individual over an enormous range, the information obtained from the expression could not be applied to a large assemblage of individuals, such as a field of wheat. This does not seem to offer a real difficulty at all. We have information now at hand to furnish the probable answer—which is that the information *could* be applied. Physiology is proceeding on a general conviction that contradicts this objection. The success of the statistical method, the success attending the use of the average in physiological work, both evidence that physiologists firmly believe that, if a sufficient number of individuals be considered, the changes in the results produced by adding more individuals will be negligible. In other words, characteristics are not variable through an infinite range, nor irregularly through a limited range. On the contrary, the distribution of characters is so ordered that by plotting numbers of individuals with a like magnitude of activity-intensity under a given set of conditions as ordinates and the corresponding magnitudes as abscissas, a curve of distribution will be obtained, a curve which has a form characteristic of the kind of activity considered and which will be unchanged by incorporating data derived from other individuals, if a sufficiently large number were considered in the first place. If, instead of the magnitude of an activity, the magnitude of the constant were thus to be plotted, there is every assurance that a curve of characteristic form would similarly be produced and, moreover, the value of the constant for the average individual could thus be determined. Naturally a sufficiently large number of individuals must be investigated, but the difficulties would be less than they now are for statistical investigation of the effect of environment upon plants, for the experiments need not be performed upon all the plants at the same time. As a matter of fact, they need not be performed under precisely the same experimental conditions, for, within limits of course, the constant characterizes the plant under all external conditions without change.

In this lies a further great advantage that will accrue to physiology. Many experiments of fundamental importance to the science are not begun because of the virtual impossibility of performing difficult or tedious operations upon a sufficiently large number of plants. In other experiments the mere collection of the required data destroys the plant. If the proper relations of the kind we are discussing were known for the physiological processes under consideration by an experimenter, many of the difficulties that hamper him would vanish. A few plants operated upon in the desired fashion would yield data that in conjunction with our plant constant would permit the extension of the conclusions to the average plant or to any individual whose constant was known. Plants need not be destroyed to ascertain the progress of some process not accessible to direct observation in the living plant, for successive measurements of a change in the externally manifested variables would permit the calculation of the corresponding changes in the internal activity.

It will be promptly objected that one of the outstanding characteristics of plants is their ability to alter their habit of living, to become really different systems, and hence to change their constants. This is actually an argument for undertaking the determination of these constants, because, whether the constant turns out to be useful or not in practical experimentation, this question of the degree of stability of inherited characteristics is fundamental to a unified science. At present plant physiology is much in the condition of chemistry before the discovery of combining weights: no theoretically valuable, quantitative experimentation was possible. Without question the greatest handicap under which plant physiology—and all biology—labors is the inability of the experimenter to evaluate the organism with which he works.

Having indicated some of the general benefits likely to accrue from the application to physiology of that clause in the physicist's creed that expresses belief in the quantitative relationships existing among the various characteristics and attributes of a material system, we may turn to an example of this clause as a means, perhaps, of making the idea more concrete. I refer to a principle proposed by Le Chatelier[3] and by Braun,[4] and often termed Le Chatelier's theorem. This principle states that all systems are conservative, or, *in extenso*, "Each change in an outer condition that affects a body or a system produces in it a change in such a direction that as a result of this change the resistance of the body or system to this outer change is increased." [5] This law, so far as physics and chemistry are concerned, is perfectly general, indeed it is embodied in the second law of thermodynamics, and there is thus additional reason for believing it to be operative in biology.[6]

Biologists long since adopted as a fundamental principle of their science what seems to be the same law stated in biological terms: they said that organisms tend to adapt themselves to changes in their environment. If an outer condition affecting the plant is altered, the plant alters within itself in such a way as to adjust its activities to the new state of affairs and to maintain recognizably its individuality.

It must now be noted that the Le Chatelier-Braun theorem implies always a connection between the directions of *two* processes that may occur in the body or system. If one of the processes is known, the theorem indicates the necessary existence and the direction of action of a second process.[7] It is with this connection that we are concerned today rather than with the theorem itself: that there is an interrelationship of plant processes which should be statable mathematically. This corollary should be as nearly universally true as the theorem itself.

It should be further noted that the "outer condition" of the theorem need not be outside the plant, for, since energy transformations occur inside the plant, portions of the plant may be "bodies or systems" in the sense of the theorem and be affected by changes in other conditions within the plant; the theorem is concerned with the energy relations of processes and their determining conditions, which are, in turn, expressions of other processes, and not with their spatial arrangements. Thus the introduction of a coil of wire between the poles of a magnet calls forth energy readjustments in accord with the theorem regardless of whether or not both coil and magnet are parts of one structural entity, a dynamo; indeed, the magnet may be excited by a part of the current induced in the coil and thus coil and magnet have part in another process. It is the thesis of this paper that these *internal interrelations of processes are denoted in biological terminology as correlations*, which are thus seen to be not biological peculiarities to be considered merely as interesting phenomena but manifestations of the operation of an ordered universe to be investigated for the light they may shed on the plant as a reactant.

---

[3] Le Chatelier, H. Sur un énoncé général des lois des équilibres chimiques. Compt. Rend. Acad. Sci. Paris **99**: 786–789. 1884.

[4] Braun, F. Untersuchungen über die Löslichkeit fester Körper und die den Vorgang der Lösung begleitenden Volum- und Energieänderungen. Zeitschr. Physikal. Chem. **1**: 259–272. 1887.

[5] Chwolson, O. D. Lehrbuch der Physik **3**: 475. Übersetzt E. Berg. Braunschweig, 1905.

[6] For discussions of this theorem from biological standpoints see Hooker, H. D., Jr. Behavior and assimilation. Amer. Nat. **53**: 506–514. 1919, and the literature there cited.

[7] Chwolson, O. D. *Loc. cit.* 475, 476.

So far the discussion has been vague with respect to the kind and number of variables that must be considered. Of course, since there are no rules to guide us, correlations and their mathematical relationships may be looked for among any characteristics of the plant. No one can say that random search would be barren of result. But since we have a somewhat extensive list of correlations already observed, which seem to have some physiological significance, it would seem to be the part of wisdom to begin with these. The Le Chatelier-Braun theorem plainly indicates a functional relation between the connected variables. A first-hand acquaintance with the general deductions of plant physiology should thus be a guide. Naturally, as the body of this sort of knowledge increases, relationships hitherto unsuspected will probably appear and, in their turn, lead to new ideas regarding the mechanisms of functional adjustment.

One must be constantly on guard, however, not to infer that use of the same term in two cases indicates real correspondence of function. The word *growth* is such a term. It is common, for example, to use dry weight of plants as a measure of growth and to use increase in size as likewise indicative of growth. Dry weight has been an elusive quantity to say the least, perhaps because it is made up of varying proportions of differently usable substances, but at any rate it would seem on purely physiological grounds that both terms should not be directly and positively indicative of the same thing. Respiration is a fundamental activity of plants. Enlargement is also. Put a plant in a situation that permits these two processes to proceed but that prevents additions of substances contributing to the dry weight, and the plant will enlarge and respire at the expense of compactness of solid substance. The more actively a plant or a tissue respires and enlarges the less compact is its solid matter. This is a matter of common observation. It would seem then that the plant's dry weight is but the material unused in respiration and enlargement and should not, by its mere magnitude, be taken as indicative of conditions favorable to the individual plant itself, although the value of dry weight to its offspring or to mankind may be large.

Regarding the number of variables to be considered, it would seem that these should be taken, not as two, as is usually done in correlation studies, but as three, for this seems to be a necessary consequence of the Le Chatelier-

Braun theorem.[8] The discovery of the third variable may furnish the key to some of those sets of graphs, the curves of which exhibit so marked a tendency toward synchrony in the occurrence of their maxima and minima—a correspondence that is evident only upon casual inspection and which vanishes upon detailed examination, leaving one with the perplexed feeling that *some* relation exists. Possibly dry weight, enlargement, and respiration may be three such variables, dry weight being considered as indicated above, although the choice of proper units will involve difficulties. This must be done, however, with individuals, not statistically, if such a relation is sought.

Thus far may we be carried by the discussion of properties of systems as a point of view. Further progress, aside from filling in the gaps in this outline, initiates the consideration of particular problems and correspondingly particular methods. There is always danger in discussing generalities and avoiding the concrete, and, lest the whole matter be thought of as still in the nebulous state of a day-dream, it may be well to state that a beginning has been made[9]—a beginning which, although modest in its comprehensiveness, seems to increase in possibilities and exactness the further it is carried. I do not present the data or the method for two reasons. First, it seems that a presentation of the general point of view would have to be made anyway and is, indeed, of vastly greater importance than a presentation of one of its applications. Leaving the mind undistracted by numerical data and their symbolic representation may allow some one to apply the general principle to problems upon which he is engaging himself, instead of inducing him to regard the matter as a problem in a field foreign to his own. The second reason is obvious: lack of time.

WELLESLEY COLLEGE,
WELLESLEY, MASSACHUSETTS

[8] Chwolson, O. D. *Loc. cit.* 476–480.

[9] Some of the early results were presented before the Physiological Section of the Botanical Society of America at its Baltimore meeting in 1918, under the title, "Growth equilibria in *Pinus Strobus*."

The Little Things That Count Big In Owning Your Own Greenhouse

Ever gone into a hat store, knowing exactly the kind and shaped hat you want; and then have some smart Alec of a salesman try to sell you the kind HE likes?

Makes a fellow madder than a wet hen, doesn't it?

Suppose, however, that same salesman had told you something about some of his hats that meant better service to you—more satisfaction for your money—you then sort of feel towards that salesman as you would an old friend.

Salesman number two is our sort when it comes to selling a greenhouse.

We do sell greenhouses, but we first sell satisfaction.

Catalog for the asking.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1922

President: H. C. Cowles
University of Chicago

Vice-President: Margaret C. Ferguson
Wellesley College

Secretary: J. R. Schramm
Cornell University, Ithaca, N. Y.

Treasurer: Irving W. Bailey
Bussey Institution, Harvard University

Councilors:

N. L. Britton
New York Botanical Garden

J. C. Arthur
Purdue University

C. E. Allen
University of Wisconsin

Any one actively interested in botanical work is eligible for membership Candidates for membership must be recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including one subscription to the American Journal of Botany. Candidates proposed for membership, and approved by the Council, may receive one copy of the Journal at $4.00 per year pending their election at the winter meeting of the Society.

Vol. IX. FEBRUARY, 1922 No. 2

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

CONTENTS

The growth of field corn as affected by iron and aluminum salts
CHARLES HOMER ARNDT 47

Control of the sexual state in Arisaema triphyllum and Arisaema Dracontium
JOHN H. SCHAFFNER 72

A demonstration of numerous distinct strains within the nominal species Pestalozzia Guepini Desm . . . . . . CARL D. LA RUE AND H. H. BARTLETT 79

Smithsonian Institution
MAR 20 1922
National Museum

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN
JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
The Thompson Institute For Plant Research, Inc.

H. H. BARTLETT,
University of Michigan

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ARTHUR H. GRAVES,
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

I. F. LEWIS,
University of Virginia

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Postage to all foreign countries, 20 cents a volume on annual subscriptions. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume, post free.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Ffty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. IX FEBRUARY, 1922 NO. 2

THE GROWTH OF FIELD CORN AS AFFECTED BY IRON AND ALUMINUM SALTS

CHARLES HOMER ARNDT

(Received for publication May 17, 1921)

INTRODUCTION

The investigations of Hoffer and Carr ('20) on corn diseases have shown that a brown or brownish-purple discoloration of the lower portion of the nodal tissue is frequently associated with evidences of malnutrition and of root rot. This discolored area they have designated zone "B." Chemical analyses showed that a high iron or aluminum content was associated with such a discoloration. The injection of iron salts produced a similar brownish discoloration, increased the catalase and oxidase activities, and reduced the H-ion concentration. Ferrous salts produced these effects more readily than did ferric salts. The injection of aluminum salts produced no discoloration, but had an effect similar to that of iron salts upon the physiological activities. Stalk- and root-rot organisms were usually associated with the accumulation of iron and aluminum in zone "B." Bordnár ('15) earlier reported a similar correlation between a high aluminum content of the sugar beet and its infection by bacterial organisms. His analyses showed that a high aluminum content preceded the infection, which indicates that an increased aluminum content is in some manner related to the decreased resistance to infection.

The present investigation was undertaken to determine whether toxic concentrations of iron and aluminum salts would produce a similar pathological condition in corn. With this object in view, a study was made of the effect of the composition of the nutrient solution upon the toxicity of sulphuric, nitric, and hydrochloric acids and of their corresponding salts with iron and aluminum.

HISTORICAL REVIEW

Some investigators of the toxicity of aluminum salts believed that the toxicity is due largely to the acid liberated in the hydrolysis of the salts. Abbott, Conner, and Smalley ('13) investigated the effect of aluminum nitrate and nitric acid on field corn. Their results indicated that nitric acid is as toxic as the same normality of aluminum nitrate. They

[The Journal for January (9: 1–46) was issued February 21, 1922.]

concluded that the toxicity is due to the hydrolysis of the aluminum salts with the accompanying liberation of nitric acid. Miyake ('16) has compared the effect on rice seedlings of the same normality of aluminum chloride and hydrochloric acid. The toxicity of these two solutions was not greatly different. The H-ion concentration produced by the acid was three times as great. This indicated that the toxicity must be due to something other than the H-ion concentration. A similar conclusion was reached by Hartwell and Pember ('18) in their study of the effect of aluminum sulphate on barley and rye. The sulphuric acid produced a H-ion concentration four times as great as the same normality of aluminum sulphate. The two substances were, however, alike toxic to barley. The acid depressed the growth of rye similarly to that of barley, but the salt had little effect on the rate of growth of rye. This indicated that plants may vary greatly in their tolerance of aluminum salts. Duggar ('11) states that the various soluble inorganic salts of the same metal are of about equal toxicity. Rothert ('06) has shown that this may not be strictly true for aluminum salts. Aluminum chloride was found to be much more toxic to corn than the sulphate. The toxicity of the salts was also dependent upon the method of application. They were most toxic when used alone in distilled water; less toxic in Knop's solution; and least toxic in soil cultures.

The toxicity of iron salts, particularly of the ferrous salts, when present in excess, is well known. Hartwell and Pember ('08) determined the effect of ferrous sulphate upon the growth of rye and barley. Katayama ('06) found that a concentration of ferrous sulphate of less than 0.01 percent stimulated the growth of barley. Higher concentrations were toxic. Clover, according to Rupprecht ('15), is seriously injured under certain conditions by ferrous salts in a concentration above 4 p.p.m. Iron hydrate when added to sand cultures is toxic to spinach. Analyses by Czadek ('04) have shown that iron in this form is easily absorbed by spinach.

Iron salts, as aluminum salts, are readily hydrolyzed. Thus, they produce an increased acidity of the sand or solution cultures. Boiret and Paturel ('92) suggested that the toxicity of ferrous sulphate is due to the acid radicle. Ferric salts are even more easily hydrolyzed and precipitated in nutrient and soil solutions. The generally recognized greater toxicity of the ferrous salts, as compared to the ferric salts, indicates that at least in the case of the ferrous salts the toxicity is possibly due to something other than the acidity. This may be due in part, as has been suggested by Awatsu ('14), to an abnormal stimulation of the physiological activities. The ferrous salts produce the greater effect on these activities. Maquenné and Demousy ('20) found that the addition of small amounts of copper sulphate to solutions containing a toxic concentration of ferrous sulphate reduced the toxicity. The addition of the copper to like solutions of ferric salts did not reduce the toxicity. They believe that the unlike effect is due to the catalytic action of the copper which hastens the oxidation of the ferrous salt to the less toxic ferric salt. The addition of calcium salts and phosphates to toxic solutions of both salts also lessened the injurious effect by aiding the formation of insoluble iron salts.

It has already been noted that most soluble iron and aluminum salts are readily hydrolyzed with an accompanying liberation of the acid radicle. Daikuhara ('14), Abbott, Conner, and Smalley ('13), Hartwell and Pember ('18), and Mirasol ('20) state that all soils which indicate injury to plants by aluminum are acid. This acidity is usually reported as the lime requirement of the soil. Recent work by Joffe ('20) indicates that this lime requirement is closely related to the H-ion concentration. Duggar ('20) has shown that the H-ion concentration may be a limiting factor in plant growth and that the effect of any particular concentration upon plant growth varies with the plant used. Good yields were secured with corn with H-ion concentrations varying from a pH of 3.2 to one of 7.1. The optimum H-ion concentration depended upon the nutrient solution and possibly to some extent upon the environmental conditions. Hoagland ('17) reported that a H-ion concentration of 0.3×10^{-3} is toxic to barley seedlings. These facts emphasize the necessity of determining the depression caused by a H-ion concentration in the nutrient solution, which lacks the toxic salt, equal to that which is produced by the hydrolysis of the iron and aluminum salts, if we wish to determine the toxicity of the salt itself. A comparison of the depression caused by the salt with that of an equal acidity produced by an acid whose anion is of little or no importance as a depressing factor, should give some indication of the toxicity of the salt itself.

Because of the importance of a suitable source of iron in nutrient solutions, it was necessary to ascertain the proper source of iron and the amount necessary to secure the optimum growth of corn. Mazé's ('19) experiments with corn lead him to recommend ferric sulphate rather than ferrous sulphate as a source of iron. Shive ('15), Tottingham ('14), and others, in the study of the salt requirements of plants, have used ferric phosphate as a source of iron. Duggar ('20) has noted that corn grown in Shive's solution becomes chlorotic. The efficiency of ferric and ferrous phosphate has been compared by Corson and Bakke ('17). They secured the best yield with the ferric salt. Shive and Jones ('21) have found lately that the use of ferrous sulphate as a source of iron in wheat cultures gives superior growth to that secured in cultures in which ferric phosphate is used. Preliminary experiments showed that ferric phosphate is not a suitable source of iron for corn in solutions of Type 1 as recommended by the Committee of the National Research Council on the Salt Requirements of Representative Agricultural Plants. Consequently, experiments were made to determine the form of iron necessary to assure a sufficient supply to prevent a lack of it from limiting the growth of the plants.

The Present Investigation

Solution cultures were used in the main part of this experimental work. These results were checked with sand cultures to determine to what extent the solution cultures might indicate the toxicity of these salts in the soil. In all cases the weight of the plants grown in the unmodified solution was taken as the control. The effect of the acid or salt is indicated as the percentage of the weight of the plants grown in solutions containing them, as compared with the control culture. The weight in the control was taken as 100 percent. For the tops the green weight was used as a criterion of the relative growth, as this probably gives the best indication of the condition of the plants. Comparisons of the green and dry weights gave similar relative weights. As a criterion of the root development, the dry weight was used. The roots were placed in weighed test tubes and dried at 100° C. for several days.

The H-ion concentration for the solutions was determined by means of the Lubs and Clark (Clark, '20) series of indicators, using the buffer mixtures recommended by them. These were carefully prepared from recrystallized salts. Standards were kept in Pyrex test tubes of 10 cc. capacity, the solutions to be tested were placed in similar test tubes, and the same concentration of the indicator was added to them. The H-ion concentration of the sand cultures at the time of the renewal of the solutions was determined by adding distilled water to bring the sand to 60 percent of its water-holding capacity; 50 cc. of the solution was drawn off by suction through a hose previously rinsed with distilled water; the new solution was then added, and the remaining 450 cc. was withdrawn. The pH value is reported for the 50 cc. and for the total 500 cc. drawn off. All solutions were clear. Consequently, turbidity did not interfere with the determinations. The pH values given to show the effect of the growth of the plants upon the reaction of the solution were determined just previously to the time of harvesting the plants. This was usually at the end of a warm, sunny period favorable for growth, as these conditions seemed to have an important effect on the results.

The seed corn used in these experiments was Reid's Yellow Dent, a variety of yellow dent (or, according to Sturtevant ('94), *Zea mays* var. *indentata*), which was furnished by Dr. G. N. Hoffer. It gave practically 100 percent germination. Very few grains showed any infection by parasitic organisms. The salts used were Baker "analyzed" chemicals. The ferric phosphate was prepared as recommended by the Committee of the National Research Council on Salt Requirements of Representative Agricultural Plants. All stock solutions of the salts and acids were made up as $N/10$ solutions. These were made up fresh at least twice a week, except the ferrous sulphate which was made up immediately before it was used. The distilled water was prepared with a Barnstead still and was stored in glass containers.

In the solution cultures, pint Mason jars and colorless cylindrical museum jars of 900 cc. capacity were used. These were treated with cleaning fluid for several hours before each experiment. The jars were completely covered with opaque black paper. A loose shell of stiff light-brown cardboard was placed over this. Flower pots were used for the sand cultures. These were thoroughly impregnated with heated paraffin and then well coated on the inside with a thin layer of the same. The sand was procured from the Whitall Tatum Company. It is the brand known as "Juniata." It was prepared as recommended by the Committee on the Salt Requirements of Representative Agricultural Plants. It had a water-holding capacity of 32 percent. It was kept at 60 percent of its water-holding capacity throughout the experiment by the method recommended by the above-named Committee.

The relative transpiration of the plants grown in the various solutions was determined from the amount of water added daily to replace the water which had been lost. The relative transpiration here reported is based on the loss for a certain period immediately preceding the time when the plants were harvested. During this period the evaporating power of the atmosphere was determined by standardized spherical white and black atmometer cups. To keep the conditions of light and temperature as nearly uniform as possible for all cultures, as no rotating tables were available, the plants were shifted daily in a systematic manner so that each plant occupied the same position for about the same period during the experiment.

Each solution culture contained four plants. Five plants were grown in each pot in the sand cultures. In most cases the cultures were run in duplicate series, so that the relative growth is based on the total weight of eight or ten plants. The weights reported in the tables are based on the mean weight of one plant.

Germination

The seed used was carefully selected for uniformity of size and shape. The seed was soaked for 15 minutes in tap water, drained, and allowed to stand at room temperature for two hours. It was next treated for 15 minutes with a 5 percent calcium hypochlorite solution (5 g. per 100 cc.). This was removed by washing the seed several times with boiled tap water. It was finally soaked for 12 hours in a shallow covered dish in just sufficient boiled water to cover the seed. After soaking, the seeds were distributed on filter paper in germinating dishes and covered with a layer of filter paper. The dish was placed in the greenhouse, covered with glass, and flooded once a day with water. When the radicles were one centimeter long, the seeds were removed to a paraffined germination net made of coarse netting. This was stretched over an inverted twenty-liter bell jar, and filled with a solution of one tenth the concentration of the solution R_2S_3

of the Type I solutions recommended by the Committee on the Salt Requirements of Representative Agricultural Plants. In addition, 2 g. of calcium carbonate was added to each jar. Tap water was used instead of distilled water. Consequently, the exact composition of this solution is not known, but as it gave excellent results, it was probably as good as any solution which can be used until we have some definite information concerning the effect of various solutions on the germination of corn. The seedlings were left on the net until the shoots were about 6 cm. long. This required from 6 to 9 days. Ten times as many seeds were soaked as were finally used. This number was reduced by one half at the time the seedlings were transferred to the net. At the time the seedlings were transferred to the solutions a second selection of the best seedlings was made. For the solution cultures these were wrapped loosely with cotton above the seed and placed in one-half-inch holes in paraffined corks. When they were to be grown in sand, the seed was placed just below the surface.

Solution Cultures

(*a*) *Solution "H."* The solution which Hartwell and Pember ('18) found satisfactory for studying the effect of aluminum sulphate on barley and rye, was found to be well adapted for the growth of corn. It was modified slightly to secure a better growth of corn. Its composition, as used, is as follows:

$CaH_4(PO_4)_2$	0.00005 *M*	$MgSO_4$	0.0008 *M*
$Ca(NO_3)_2$	.0015 *M*	$Al_2(SO_4)_3$	.000003 *M*
NH_4NO_3	.001 *M*	$MnSO_4$	.00001 *M*
KCl	.0008 *M*	$ZnSO_4$	.000005 *M*
		Total	0.004168 *M*

To this was added 7 mg. of $FePO_4$ per liter. For convenience, this solution will be referred to as solution "H." Preliminary experiments indicated that Hartwell's solution gave the best results when aluminum, zinc, and manganese were added. The work of Mazé ('15) on the salt requirements of corn suggested this modification. The phosphate concentration of the solution used is about twice that recommended by Hartwell and Pember. There was no sign of precipitation when aluminum salts were added and the solution was allowed to stand a week. Again, all solutions were changed every other day, or at one half the interval used by Hartwell and Pember. The ferrous-sulphate solutions were changed every day to prevent, as far as convenient, the change from the ferrous to the ferric condition. It was impossible to prevent precipitation when the ferric salts were used. Consequently, the results may not truly represent the relative toxicity of the ferrous and the ferric salts.

(*b*) *Solution "A."* A number of preliminary experiments demonstrated that with solutions of Type I as recommended by the Committee on the

Salt Requirements of Representative Agricultural Plants, a solution of one half the molecular concentration of R_2S_3 was well within the range necessary for the optimum growth of corn. A solution was used whose partial volume-molecular proportions were as follows: KH_2PO_4, 0.0024 *M*; $Ca(NO_3)_2$, 0.0036 *M*; $MgSO_4$, 0.0035 *M*. This solution probably had an osmotic pressure close to 0.5 atmosphere.

In this solution it was impossible to prevent precipitation when the various salts were added. Consequently, the results of the experiments in which this solution was used are not strictly comparable with those in which solution "H" was used. They are comparable when considered in relation to the effect of the composition of the nutrient solution upon the tolerance of acidity by corn. Because of this precipitation, a renewal of the solutions at small intervals would have had little effect on the amount of the salt in the solution. To economize time, the ferrous sulphate solutions of 0.001 *N* and higher were changed twice a week; the others were changed once a week.

Sand Cultures

Solution "H" was used in all sand cultures. The method of McCall ('16) as modified by Johnson ('20) was used for changing the solution. The cultures containing ferrous sulphate were renewed every day; the others, every third day. The sand did not contain sufficient iron for the optimum growth of corn. Ferric phosphate was added to the solution as in the solution cultures. The ferric phosphate was added to the ferrous-sulphate cultures every third day, as most of the phosphate probably was held in the sand and was not withdrawn when the solutions were renewed.

After 2000 g. of sand was placed in each pot, it was thoroughly washed a second time by drawing 3 liters of distilled water through it. The sand used for the acid cultures was treated for 48 hours with strong sulphuric acid and then thoroughly washed until the water withdrawn was neutral. These sand cultures were not covered with a wax seal. This was thought desirable in order to simulate soil conditions as far as possible. A wax seal would have reduced the aëration and induced conditions in the soil which would undoubtedly have increased the toxicity of the ferrous salts. In order to secure some idea of the relative amount of water lost through evaporation and transpiration, a pot without plants was placed in the series and treated as the others. The amount lost through transpiration was calculated by deducting the loss from this pot from the total loss of the other cultures. The error introduced by this method of determining the transpiration should be small, as the loss through evaporation was relatively small in comparison to the total water loss.

The writer wishes to express to Dr. J. W. Harshberger his sincere appreciation of the interest and assistance which made possible the experiments, and to acknowledge his indebtedness to Dr. G. N. Hoffer of the Office of

Cereal Investigations of the United States Department of Agriculture for the suggestion of the problem and for much valuable information. Dr. Alice M. Russell has added greatly to the interest of the investigation by her mycological study of the discolored nodes. To Dr. R. H. True and to other members of the Botanical Department of the University of Pennsylvania, the writer is greatly indebted for generous help and criticisms.

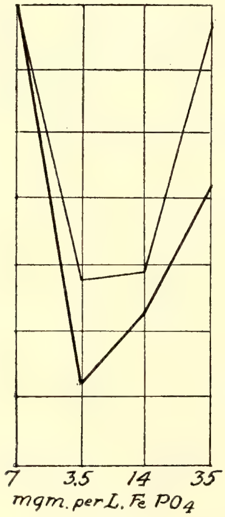
TABLE I. *The Effect of the Composition of the Nutrient Solution upon the Availability of Iron in Ferric Phosphate*

Solution	Mg. $FePO_4$ per Liter	Total Weight		Relative Weight	
		Tops	Roots	Tops	Roots
"H".........	7	82.6 gm.	3.84 gm.	100%	100%
"A".........	3.5	34.4 "	2.24 "	42%	58%
"A".........	14	43.9 "	2.3 "	53%	59%
"A".........	35	59.84 "	3.68 "	72%	97%

EXPERIMENTAL DATA

I. The Effect of the Composition of the Nutrient Solution Upon the Availability of Iron in Ferric Phosphate

Four cultures were set up in duplicate as shown in table 1. The plants were grown in the 800-cc. jars during the period from February 7 to March 5, 1921. The "H" solutions were changed twice a week; the others every



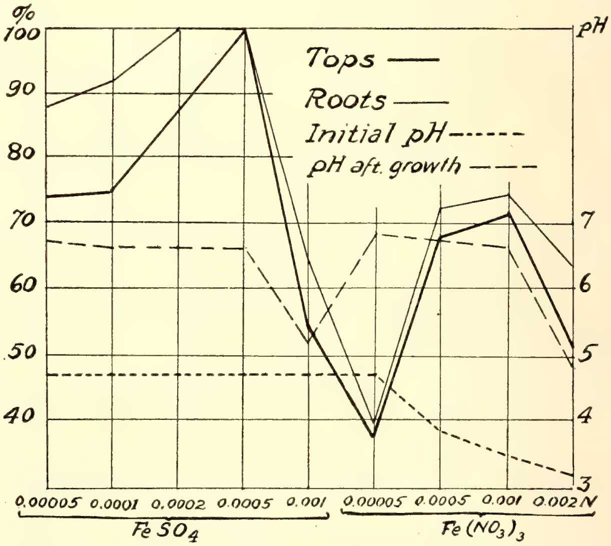


FIG. 1 (left). Relative growth of roots and tops with $FePO_4$ in solution "A" (3.5, 14, 35) and "H" (7).

FIG. 2 (right). Relative growth in solution "A" with $FeSO_4$ and $Fe(NO_3)_3$. Also H-ion concentration before and after growth.

week. All the plants in solution "A" were unquestionably chlorotic, which fact indicated that they did not secure sufficient iron. The plants grown in solution "H" were of a normal color. Other experiments showed that an increase of iron in this solution did not increase the yield. An increase in the amount of ferric phosphate in solution "A" did increase the yield. The color of the plants, however, indicated that the iron was not available in sufficient quantities. Plate IV and also figure 1 show clearly the difference of growth in the various concentrations.

II. The Effect of Iron Salts in Solution "A"

Two preliminary experiments, in which ferrous sulphate was used as a source of iron, were made in the fall of 1920. This was a poor period for growth because of the great amount of cloudy weather. The results are of interest in comparison with those of the experiment performed under more favorable conditions during March, 1921. In this experiment, the plants were grown for 25 days in 800-cc. jars. The first two experiments will be referred to in table 2 as series 1 and 2 respectively; the latter as series 3. The relative transpiration is reported for the last week of growth for series 3. During this period the average daily loss from the white and the black atmometer was 11.4 cc. and 13.6 cc. respectively.

TABLE 2. *The Effect of Iron Salts upon the Relative Yields of Tops and Roots and upon Transpiration in Solution "H"—Also the Initial pH and the pH After Growth*

Salt	Normality	Yield of Tops			Yield of Roots	Transpiration	pH			
							Initial	After Growth		
		Ser. 1	2	3	3	3		1	2	3
0.00		35%					4.7	5		
$FeSO_4$	0.00005			74%	88%	81%	"			6.7
"	.0001			75	92	75	"			6.6
"	.0002		66%	88	100	84	"		5.6	"
"	.0005	100	100	100	100	100	"	5.5	"	"
		(2.6 gm.)	(4.21 gm.)	(24.7 gm.)	(0.48 gm.)	(790 cc.)				
"	.001	68	66	54	67	52	"	5	5.2	5.2
"	.002	62	63				"	4.3	4.1	
"	.004	47					"	4		
"	.008	21					"	3.5		
$Fe(NO_3)_3$	.00005			37	38	38	"			6.8
"	.0005			68	73	70	3.9			6.7
"	.001			71	74	71	3.5			6.6
"	.002			51	63	53	3.2			4.8

All plants grown in the solutions containing ferric nitrate were chlorotic. This fact, and the low yields associated with it, indicated that ferric nitrate was much inferior to ferrous sulphate as a source of iron in solution "A." In all cases, the best yield was secured with 0.0005 *N* ferrous sulphate. The above-noted results for series 3 are shown graphically in figure 2. A comparison of figures 1 and 2 shows that the lack of iron does not usually depress root development as much as it does that of the tops. It is interesting to note the poor root development in 0.0005 *N* ferric nitrate. There is little difference in the relative growth with either salt in 0.001 *N* and 0.002 *N*. It is singular that ferric nitrate should begin to depress growth before it supplies sufficient iron for normal growth.

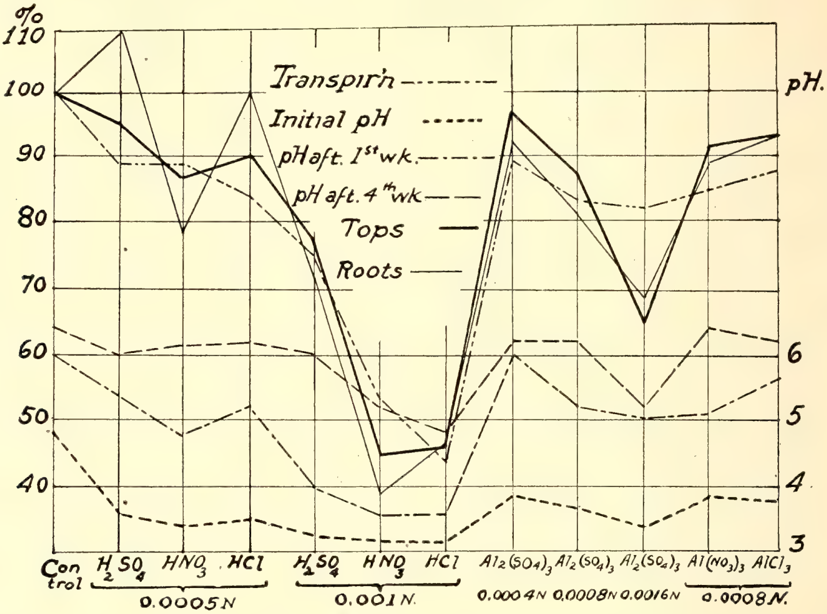


FIG. 3. Relative growth and the H-ion concentration upon the addition of acids and aluminum salts to solution "A." The H-ion concentration is given for the initial solution and at the end of the first and last weeks of growth.

TABLE 3. *The Effect of Acids and Aluminum Salts in Solution "A"*

Sol. "A" plus	Normality	Rel. Weight		Rel. Transpiration	pH		
						After Growth	
		Tops	Roots		Initial	1st wk.	4th wk.
0.00		100% (10.1 gm.)	100% (0.24 gm.)	100% (560 cc.)	4.8	6	6.4
H_2SO_4	0.0005	95	105	89	3.6	5.4	6
"	.001	77	73	75	3.3	4	6
HNO_3	.0005	87	77	89	3.4	4.8	6.2
"	.001	45	38	53	3.2	3.6	5.2
HCl	.0005	90	100	84	3.5	5.2	6.2
"	.001	46	38	44	3.2	3.6	4.8
$Al_2(SO_4)_3$	.0004	98	93	89	3.9	6	6.2
"	.0008	87	81	83	3.7	5.2	6.2
"	.0016	65	69	82	3.4	5	5.2
$Al(NO_3)_3$	.0008	91	89	85	3.9	5.2	6.4
$AlCl_3$	.0008	93	98	88	3.8	5.6	6.2

III. The Effect of Aluminum Salts and Acids Upon Growth in Solution "A"

The object of this series of experiments was to determine the relative toxicity of hydrochloric, nitric, and sulphuric acids and their corresponding aluminum salts. The solution was modified by the addition of the acids and the salts as shown in table 3. As previously stated, the aluminum salts were precipitated in this solution, and the concentration added does not represent the amount remaining in solution. The results, consequently, do not represent the real relation between the toxicity of the acid and that of the salts. The plants were grown for 30 days during the month of April in a shaded greenhouse. Pint Mason jars containing 400 cc. of the culture solutions were used. The results are shown graphically in figure 3. The weather was very favorable during the first portion of the period. There was practically no sunshine during the last week. This fact, undoubtedly, accounts for the small change in the pH value for the last week of growth.

TABLE 4. *The Effect of Acids and Iron and Aluminum Salts upon Growth in Solution "H"*

Sol. "H" plus	Normality	Yield of Tops		Yield of Roots 2	Transpiration 2	pH		
		Ser. 1	2			Initial	After Growth	
							1	2
Control		100% (17.5 gm.)	100% (20.2 gm.)	100% (0.58 gm.)	100% (745 cc.)	4.9	4.9	6.2
H_2SO_4	0.0002	97				3.7	4.6	
"	.0004	71	78	71	74	3.5	4.3	6.2
"	.0006	88	67	65	69	3.2	3.5	6
HNO_3	.0002	90				3.7	4.6	
"	.0004	82	76	69	68	3.4	4.3	6
"	.0006	68	62	55	51	3.2	4.5	5.6
HCl	.0002	97				3.7	4.3	
"	.0004	82	88	71	84	3.5	3.7	6.2
"	.0006	69	63	53	49	3.2	3.4	5.4
$FeSO_4$	.0002	72	71	66	60	4.6	4.8	4.4
"	.0004	62	52	54	50	4.6	4.6	3.8
"	.0006	41				4.2	4.3	
$Fe_2(SO_4)_3$	.0002	100				4	5	
"	.0004	72	67	66	52	3.7	4.3	4.7
"	.0006	75	73	61	67	3.5	3.5	4.7
$Fe(NO_3)_3$	.0002	97				4	4.3	
"	.0004	69	62	65	54	3.7	4.3	4.6
"	.0006	52	64	57	59	3.5	4.3	4.7
$FeCl_3$	.0002	95				4	4.2	
"	.0004	86	63	61	56	3.6	4.3	4.7
"	.0006	67	51	51	54	3.5	3.5	3.3
$Al_2(SO_4)_3$	.0001	91	88	88	84	4.2	4.8	5.8
"	.0002	84	74	73	66	4.1	4.7	4.8
"	.0004	73	62	59	52	3.9	4.2	4.2
"	.0006	64	57	67	53	3.8	3.9	4
$Al(NO_3)_3$	.0002	63	66	67	63	4.2	4.7	4.6
"	.0004	53	57	50	52	4.2	4.3	3.7
"	.0006	50	53	48	45	4	4.1	4.2
$AlCl_3$	.0002		73	74	66	4.2		4
"	.0004		64	62	52	4.1		3.9
"	.0006		64	60	49	3.9		3.5

The pH value at the end of the first week is also given. The water loss by transpiration is given for the entire growth period. The average daily loss from the white and black atmometers was 5.83 cc. and 6.49 cc., respectively.

IV. The Effect of Iron and Aluminum Salts Upon Growth in Solution "H"

(*a*) *Solution Cultures.* As solution "H" has been previously described, only a brief account of the experiment and the environmental conditions will be given here. The pint jars and 450 cc. of the culture solutions were used in all cases. Series 1 was run for 37 days during January and February. Conditions for growth at the beginning of the experiment were not very favorable. Conditions were very favorable for growth during the 37-day period in which series 2 was grown. The plants grew rapidly and seemed to be in excellent condition until several days before the end of the experiment, when the leaves of some of the plants were unable to open because of the formation of a mucilage-like substance. This was most noticeable in the solutions containing aluminum. This pathological condition was associated with a number of bright sunny days during which it was impossible to keep the greenhouse temperature below 32° C. It never exceeded 35° C. The relative transpiration is given for series 2 and is based upon the transpiration for the last week of growth. During this period the average daily loss from the white and the black atmometers was 11.4 cc. and 13.6 cc., respectively.

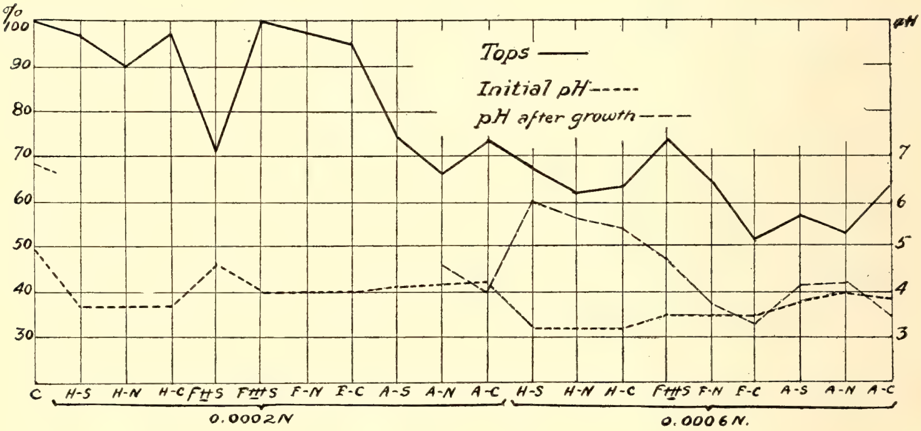


FIG. 4. Relative weight of tops and H-ion concentration before and after growth, in solution "H" as they were affected by the addition of acids and salts in 0.0002 *N* and 0.0006 *N* concentration. In this and the following figures, the following notation is used: *C*, control; *H*, *F*, and *A* denote the cations H, Fe, and Al. The anions are indicated thus: *S* = SO_4; *N* = NO_3; *C* = Cl. Ferrous and ferric sulphate are distinguished by plus signs; F^{++} *S* = ferrous sulphate, F^{+++} *S* = ferric sulphate.

(*b*) *Sand Cultures.* The plants in the sand cultures were grown for 40 days in April and May. The modifications of solution "H" and the

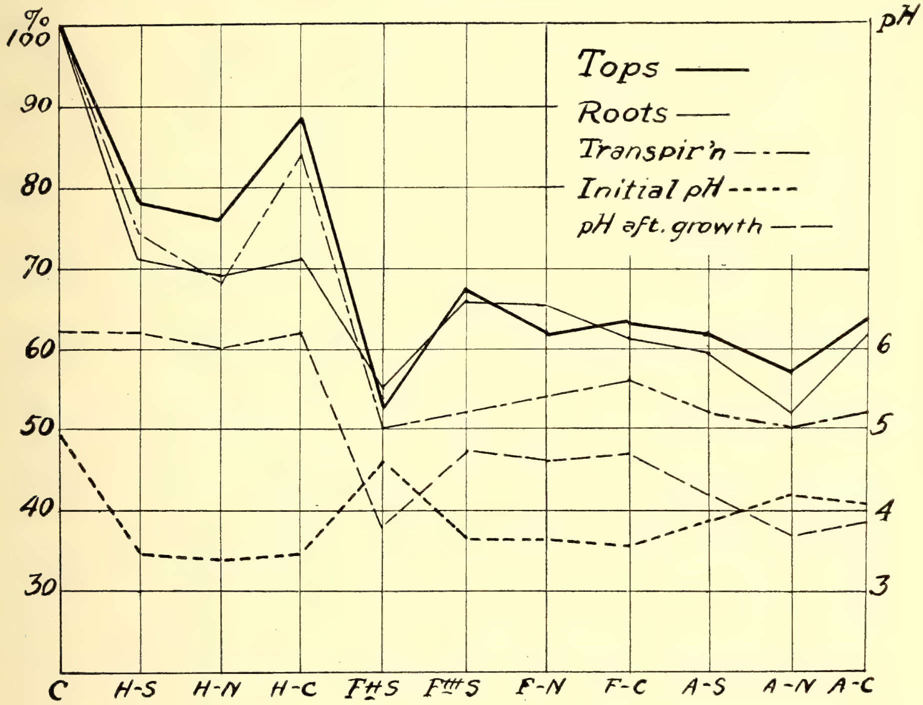


FIG. 5. Relative growth of tops and roots, transpiration, and the H-ion concentration before and after growth in solution "H" upon the addition of 0.0004 *N* acid and salts. For notation see figure 4.

results are given in table 5. The transpiration is reported for the last 16 days of growth. The total average loss by evaporation from the pots without plants, which was deducted from the total loss of the others to determine the amount lost by transpiration, was 405 cc. During the period of growth the average daily loss from the white and black atmometers was 7.83 cc. and 8.5 cc. respectively. The small difference between these values is due to the fact that the plants were grown in a well shaded greenhouse and to the large proportion of cloudy weather during the first part of the experiment. On sunny days the temperature ranged from 30° C. to 35° C. This was probably too high for the optimum growth of corn. The pH values are given for the first 50 cc. and for the total 500 cc. drawn off at the end of the second week, and also at the time of the last renewal for which the pH value of the last 50 cc. is also given. The change in the pH values of the initial solutions by plant growth was much greater in the sand cultures than in the solution cultures, as is well shown in figure 6. The depression of growth of the tops is also on an average less, and particularly so in case

TABLE 5. *The Effect of Acids and Iron and Aluminum Salts upon Growth in Sand Cultures*

Sol. "H" Plus	Normality	Weight of Tops	Root Score	Transpiration	pH					
					Initial	End 2d wk.		End last wk.		
						50 cc.	500 cc.	1st 50 cc.	500 cc.	Last 50 cc.
Control		100% (12.8 gm.)	21	100% (1410 cc.)	4.9	6.2	6.2	6.4	6	6
H_2SO_4	0.001	91	16	100	3	5.4	3.8	5.6	3.8	3.6
"	.002	59	8	52	2.8	3.4	3	3.4	3	3
HNO_3	.001	101	14	98	3	5	3.8	6	4	3.6
HCl	.001	89	11	86	3	5.4	3.8	5.6	3.6	3.6
$FeSO_4$	.0002	102	23	99	4.6	5.8	5.6	4.8	4.6	4.6
"	.0004	88	2	79	4.6	5.6	5.6	4.4	4.2	4.2
"	.0006	49	0	60	4.2	3.8	3.6	3.4	3.4	3.6
$Fe_2(SO_4)_3$	.0004	79	10	75	3.7	5.8	5.8	5.8	5.2	5
$Fe(NO_3)_3$	.0004	80	8	69	3.7	5.6	5.8	5.2	5	4.8
$FeCl_3$	.0004	66	6	61	3.7	6	5.8	5.6	5	4.8
$Al_2(SO_4)_3$	.0002	69	4	70	4.1	6	6	6.2	6	5.8
"	.0004	66	3	69	3.9	5.8	5.8	5.8	5.2	4.8
" [1]	.0006	57		58	3.9			4.1	4	
$Al(NO_3)_3$	.0002	73	6	74	4.2	6	6	6.2	6	5.8
"	.0004	54	5	64	4.2	6	6	5.4	4.8	4.8
" [1]	.0006	54		58	4			4	5	
$AlCl_3$	.0002	80	9	76	4.2	5.8	6	6	5.8	5.4
"	.0004	63	1	67	4.1	6.2	6.2	5.4	4.8	4.8
" [1]	.0006	56		48	4			4	4	

of the peculiar behavior of ferrous sulphate at 0.0002 *N* and 0.0004 *N*. This difference is probably accounted for by the better aëration of the sand cultures. It is still more unusual to have the lower concentration give a slightly higher yield than the control, and 0.0004 *N* a higher yield than the same concentration of the ferric salts. This is probably to be explained by the fact that the ferrous solutions were renewed every day; the others every 3 days. The addition of sand to the culture solution changed greatly the toxicity of the acids. The average depression in the sand cultures for 0.001 *N* acid was 6 percent, and in the solution cultures for 0.0004 *N* it was 21 percent.

The aluminum salts produced approximately the same depression in both types of cultures. The corresponding average depressions in the sand and solution cultures for 0.0002 *N* are 26 percent and 28 percent, and for 0.0004 *N*, 39 percent and 40 percent, respectively. The extensive researches of Shive ('20) have indicated that this similarity in effect in sand and solution cultures is to be expected when the solutions are changed at frequent intervals, and that such factors as chemical action and precipitation must not be considered.

The inhibiting effect upon root development of the acids and of the iron and aluminum salts was much less in the sand than in the solution cultures. A 0.0006 *N* concentration of ferrous sulphate in the sand cultures inhibited their development relatively less than 0.0002 *N* in the solution cultures, 0.001 *N* H_2SO_4 less than 0.0006 *N;* and 0.0004 *N* of the aluminum salts much less than 0.0002 *N*. In the solution cultures the dry weight was used as a criterion of relative root growth. The results, for reasons to be discussed later, were not satisfactory. In this experiment the roots were washed free of sand and then compared by the method devised by Free ('15). A comparison can readily be made by reference to the numbers reported. The highest number indicates the best root development.

---

[1] These results are taken from a series run previous to the time the other cultures were grown.

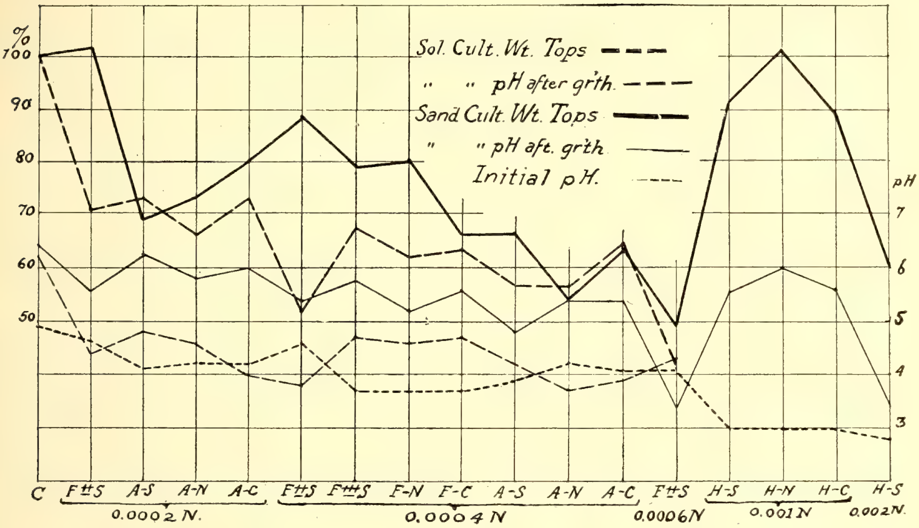


FIG. 6. Relative growth in sand and solution cultures and the effect of plant growth upon the H-ion concentration. For notation see figure 4.

DISCUSSION

It is plainly evident from the experiments described in the preceding pages that the effect of any particular salt upon plant growth depends largely upon the composition of the solution in which the plants are grown. One of the best examples is the difference in the availability of the iron in ferric phosphate in solutions "A" and "H." Something in solution "A" prevents the plant from absorbing the iron. This is not due to any inherent property of the ferric phosphate. At the time series 2 of experiment 4 was run, plants were grown in a modified solution "H." The calcium phosphate was replaced by an equivalent molecular weight of ferric phosphate. The growth of the tops in these cultures relative to the control was 95 percent; of the roots, 68 percent, and the transpiration was 96 percent. The plants were as well developed as those of the controls. The lower nodes of these plants did not show any discolored nodes, such as were found when the plants were grown in the solution with this concentration of the other iron salts. This behavior of ferric phosphate was very interesting when it formed as a precipitate when ferric nitrate was added directly to solution "A." A 0.001 *N* concentration did not produce plants of a normal green color. A 0.002 *N* concentration was decidedly toxic. It is singular that growth should be depressed by the addition of an iron salt, before sufficient iron is available for the growth of the plant. When the efficiency of ferric nitrate as a source of iron is compared with that of ferrous sulphate, the result is striking. At 0.0005 *N*, the yield in the latter is 68 percent of that in the former; while at 0.001 *N*, the ferrous salt becomes very toxic and the ferric salt gives the better yield. This greater physiological activity of the ferrous salt may be correlated with its greater influence on catalytic activities. Other factors, however, may be concerned. The ferrous salt is less readily precipitated than the ferric. There may also be a difference in the solubility of the two phosphates which may be influenced by the concentration of phosphorus and calcium in the solution. Any attempt at an explanation of this difference can be only speculative until more data are available.

The concentration of ferrous sulphate necessary to give the maximum yield with corn in solution "A" is probably close to 0.0005 *N*. This is approximately 14 mg. per liter. The iron requirements of corn are evidently very different from those of wheat and probably also from those of rice as determined by Shive and Jones ('21) and Gile and Carrero ('20). The findings of Corson and Bakke ('17) apparently are not applicable to corn. It is somewhat difficult to interpret Mazé's recommendation of a ferric rather than a ferrous salt. He used a concentration of 50 mg. of ferrous sulphate per liter, an amount which would have been extremely toxic in solution "H." Mazé ('13) noted that everything depends on the relative proportion of all elements. This is well illustrated by the behavior of ferrous sulphate in solutions "H" and "A." A concentration of it in solution "H," two fifths of that required for optimum growth in solution "A," reduced the yield 25 percent to 30 percent. A doubling of this concentration in solution "H" reduced the yield almost 50 percent. The three ferric salts are about alike toxic, and their effect upon growth in solution "H," when compared to ferrous sulphate, is practically of the same order as the relative efficiency of ferric nitrate and ferrous sulphate in promoting growth in solution "A." At a 0.0002 *N* concentration they are practically without effect upon the yield; and at 0.0004 *N*, they gave about a 15 percent better yield than the ferrous salt.

The toxicity of aluminum salts at the higher concentrations does not seem to be a function of the concentration. The average depressions are: 0.0001 *N* 10 percent, 0.0002 *N* 28 percent, 0.0004 *N* 40 percent, 0.0006 *N* 42 percent in the solution cultures, and 0.0002 *N* 26 percent, 0.0004 *N* 39 percent in the sand cultures. There is an increase in toxicity of aluminum salts, as measured by yield of tops, with increasing concentration; but it is not at all proportional to the increase in concentration. The effect on the development of the roots is more nearly related to the concentration. This difference in root development is much greater than is actually indicated by the small differences in the relative root yields. The development of the small secondary roots is inhibited at the higher concentrations; but coinciding with the stunting of the secondary roots there is a thickening of the main roots and an increased formation of prop roots, which increases the weight of the root system. Because of this fact, the dry weight is a poor criterion of the root development and of the number of feeding roots. In the solution cultures, the roots were barely able to penetrate a solution of 0.0006 *N*, no secondary roots formed, and the root tips were frequently swollen and recurved. Nevertheless, the roots were able to absorb sufficient material to produce a stunted growth. The poor development of the roots in the higher concentration probably accounts to some extent for the tendency of chlorosis to be associated with aluminum injury. It is likely that the capacity of aluminum salts to antagonize the action of iron salts, as has been noted by Stoklasa ('18*b*), may be the most important factor.

The effect of aluminum salts on root development described above is similar to that noted by Rothert ('06) in his study of the effect of aluminum chloride and sulphate upon the growth of *Zea mays*. He states that the shoots suffer the least and the secondary roots the most. A 1 percent solution was necessary to depress growth in Knop's solution, which is many times the concentration necessary to depress growth in solution "H." Hartwell and Pember ('18) secured a 44 percent depression of the growth of barley with 0.0008 *N* aluminum sulphate in solution "H." It is very likely that this concentration would not produce a much greater depression with corn.

Some interesting suggestions as to the possible cause for this peculiar action of aluminum salts may be found in the studies of Stoklasa ('18*a*, *b*), Berthelot and André ('95), and Meurer ('08). The latter has shown that the amount of aluminum absorbed by slices of beet or carrot roots from a solution of one of its salts is independent of the concentration of the salt in the solution. Tissue killed by chloroform absorbs the aluminum as readily as living tissue. He concludes that aluminum forms some chemical compound with the pectic bodies of the middle lamella. The relatively lesser toxicity of aluminum salts at increased concentrations has led Stoklasa ('18*b*) to conclude that aluminum combines with the cell wall to form colloidal salts. This action renders the cell less permeable. The researches of Szücs ('12) demonstrated that it also acts on the protoplasm and causes it to set. The singular thickening of the main roots of the plants grown in the solution cultures is probably due to some unique action of the aluminum upon the tissues of the root. Berthelot and André grew plants in pot cultures to which aluminum salts were added. In all the plants grown in their cultures, aluminum oxide formed a much larger percentage of the ash of the roots than of the tops. They concluded that aluminum after absorption is fixed and thus rendered immobile. Stoklasa ('18*a*) has shown that the amount of aluminum in the roots of mesophytic plants is determined largely by the amount of soil moisture. An increase in the soil moisture increased the percentage of aluminum in the roots, but not in the tops in which the percentage was always low. These facts probably explain the lessened depression of root growth by the aluminum salts in the sand cultures. Rothert's analyses of the corn plants which he grew in the cultures containing aluminum showed a large percentage of aluminum in the ash of the roots and only a trace in the tops. Hoffer and Carr ('20) have shown that most of the aluminum which is carried to the stem accumulates in the nodal area. Microchemical tests made with plants grown in the present experiment confirm this finding.

In the present investigation no evidence has been found to confirm Rothert's statement that aluminum chloride is more toxic than aluminum sulphate. There is some indication of a slightly greater toxicity of the aluminum nitrate.

The H-ion concentrations of solutions "A" and "H" are slightly below those which Duggar ('20) has given as the optimum for corn. The slight depression of growth caused by 0.0002 N concentration of H_2SO_4 in solution "H," by 0.0005 N in solution "A," and by 0.001 N in the sand cultures, indicates that an initial acidity above pH 3.7 in solution "H," above pH 3.6 in solution "A," and above pH 3.6 in the sand cultures had little effect upon the growth of field corn. When the initial concentration of the acid in solution "H" is increased to 0.0004 N (pH 3.5), there is a distinct depression which is greater than that caused by 0.001 N (pH 3) in the sand cultures. A 0.0006 N concentration of nitric, hydrochloric, or sulphuric acid is decidedly toxic in the solution cultures. The three acids produced a similar depression of the relative weight of tops and roots. The roots were much better developed in the sulphuric acid solutions, when compared with the effects of the other acids, than is indicated by the relative weights. A similar difference was noted in the solutions containing the three ferric salts. This feature is clearly shown in Plate IV, figure C. The difference in development is in harmony with the favorable effect of sulphates on the root development of certain plants which has been noted by Tottingham and Hart ('15). In solution "A," as shown in figure 3, the sulphuric acid gave a better yield of the tops than did the other acids. This is most marked with a concentration of 0.001 N. The relative yields reported in this paper, indicating the effects of the various concentrations of the acids in solution "H" on corn, are very similar to those given for wheat, barley, and oats by Hartwell and Pember ('07).

It will be noted from the tables and figures that the solutions in general tended to become less acid with the growth of the plants. A similar tendency has been noted by Duggar ('20), Salter and McIlvaine ('20), and

Hoagland ('19). The acid solutions were more readily shifted toward neutrality than the solutions containing the iron and aluminum salts. There seems to be a tendency for the sulphuric acid solutions to be shifted most readily, particularly in solution "A." The effect of the acid radicle of the aluminum salts in determining the shifting of the reaction is shown by a comparison of the initial H-ion concentration and the concentration after growth. This is well shown by the various graphs. The sulphate solutions, with the exception of those containing ferrous sulphate, are unique in that in all cases the H-ion concentration was shifted toward neutrality. The reverse was true of the solution cultures containing ferrous sulphate even at the low concentration of 0.0002 *N*. All solutions containing nitrates become less acid except 0.0004 *N* aluminum nitrate. The chlorides showed a strong tendency to increase the initial H-ion concentration. This tendency was shown by all concentrations of aluminum chloride in solution "H," and also by 0.0006 *N* ferric chloride. In the sand cultures the 0.0006 *N* ferrous sulphate solution was the only one which showed after plant growth a H-ion concentration higher than the initial concentration of the solution.

The results of Salter and McIlvaine show that a H-ion concentration less than that necessary to depress growth in solution "H" may produce a distinct depression in other nutrient solutions. They found that a reaction of pH 4.11 was much less favorable to corn than a reaction of pH 5.16, in their strongly buffered solutions. The great changes in the reaction of solutions "A" and "H" produced by the growth of corn indicate that possibly the initial concentration of the solution is not as important a factor in plant growth as is the buffer action of the solution which determines the ease with which the plant can shift the reaction. It is very likely that the toxicity of the acids in solutions "A" and "H" could have been increased either by using larger amounts of the solutions, or by renewing them more frequently. A survey of the tables will show that the shifting of the reaction is roughly proportional to the size of the plant. Thus, the pH values given in table 3 and figure 3 show that the change in the reaction is greater at the end of the fourth than at the end of the first week; and in table 4 it appears that the series (ser. 2) which gave the greatest total growth also produced the greatest change in reaction.

In solutions containing ferrous sulphate, growth shifted the H-ion concentration in the opposite direction. The change was almost negligible at the beginning; but as the plants became larger, the H-ion concentration was increased. A solution with a similar concentration of the salt was kept in the greenhouse in a flask for three days without a change equal to pH 0.2. It seems fairly evident that the plant in some manner accelerates the hydrolysis and the precipitation of the salt and is the main agency which produces the change in acidity. The ferric salts are immediately precipitated, giving a high initial acidity to the solution. The ferrous salt is less readily precipitated, but when conditions are such as to favor its precipitation a similar acidity is produced.

A comparison of the relative yields in solution "H," as shown in figures 4, 5, and 6, of the acids and the aluminum salts, points to the cation of the salt rather than the H-ion concentration produced by the hydrolysis of the salts as the toxic factor. The reverse is true in solution "A" (fig. 3). The results in solution "H" indicate that corn is affected by soluble aluminum salts similarly to rice (Miyake, '16) and barley (Hartwell and Pember, '18; Conner, '21). A closer relation seems to exist between the ability of the plant to shift the reaction toward neutrality and the relative toxicity of the acid and the salt, than exists between the initial H-ion concentration and the toxicity of the salt. This inability of the plant to change the reaction may be a secondary feature, for, as has been previously pointed out, this change is related to the amount of plant growth and it may be merely a measure of the toxicity of the solution rather than the cause of the toxicity. These results are not in harmony with the conclusions of Abbott, Conner, and Smalley ('13). These authors based their conclusions largely upon the root development of seven-day-old seedlings. Plate IV, figure B, which shows the relative development in 0.0004 *N* acid and in the same normality of the corresponding aluminum salts, indicates that there is a distinct difference between the toxicity of the acid and that of the salts as measured by root development when a longer period of growth is used. These results agree with those of Miyake. He based his conclusions on the relative elongation of the shoots and states that there is little difference in the toxicity of the same strengths of the acid and of the salt. His results on root elongation show that the root growth in the acid was twice as great as in the corresponding salt solution. The relative toxicity of the acid and the salt in solution "H" depends somewhat on the concentration used. Except in the case of sulphuric acid, there is little difference in the toxicity of the acid and of the corresponding salt in a 0.0006 *N* concentration; while at 0.0002 *N*, the acid has practically no effect upon growth and the salt produces a considerable reduction of growth. A comparison of the relative effects of the acid and of the salt, as shown by the results given for the sand cultures in table 5 and in figure 6, is even more favorable to the acid. These results are also more dependable when considered in relation to the H-ion concentration, as the buffer action of the sand kept the concentration more constant; although it is possible that the concentration reported does not represent the acidity of the solution in contact with the roots. In these cultures a 0.002 *N* concentration of sulphuric acid in the culture was necessary to produce the same depression as 0.0006 *N* aluminum sulphate. The difference in H-ion concentration is still greater. In the acid cultures it was approximately four times as great, as marked by the difference between pH 3.4 and pH 4.1. This indicates that the theory advanced by Abbott, Conner, and Smalley, which states that the toxicity of aluminum nitrate toward field corn is largely due to the acidity produced by the hydrolysis of the salt, is no longer tenable. It has been previously noted that all soils which show aluminum injury are acid. A comparison of the pH values reported at the end of the second and at the last renewal (5th week) in the sand cultures shows an increase in acidity of the cultures by the continued addition of aluminum salts, which fact supports the belief that the hydrolysis of aluminum salts may be an important factor in the production of soil acidity through the reactions noted by Noyes ('19).

Hartwell and Pember ('08, p. 293), after their investigation of the effect of the same normality of sulphuric acid and ferrous sulphate on barley and rye, conclude that the injury caused by the salt is largely due to the liberation of the acid radicle in hydrolysis. A comparison of the relative growth which they report for 0.0004 *N* ferrous sulphate and the growths given here in table 4 shows that this is not the case with corn in solution "H." A 0.0002 *N* ferrous-sulphate concentration depressed the growth almost 30 percent, but it did not produce an acidity greater than pH 4.4. Since the H-ion concentration of the solution used by Hartwell and Pember is not stated, it is somewhat difficult to compare their results with those of the present experiment. A still greater difference will be noted in the sand cultures where a 0.0006 *N* concentration of ferrous sulphate produced a 10 percent greater depression than 0.002 *N* sulphuric acid. The H-ion concentration of both cultures is the same. The score given in table 5 to indicate the relative root development is very favorable to the acid solution. The cultures in solution "A" show most clearly that the toxicity of ferrous sulphate is in no way related to an increase in acidity. The 0.001 *N* ferrous sulphate culture never showed a H-ion concentration below that of the initial solution, but it did produce a 35 percent to 45 percent reduction of growth.

The toxic effects of the ferric salts in the solution cultures were more nearly related to the H-ion concentration than was the case with either the ferrous or the aluminum salts. This is because they are immediately precipitated. Thus, they produce a high initial H-ion concentration. In the sand cultures the 0.001 *N* acids gave a far better yield than a 0.0004 *N* concentration of the iron salts. A comparison of the acids and the ferric salts in these cultures is probably not permissible because the rapid precipitation of these salts probably caused a much higher concentration of iron in the sand cultures than is indicated by the original solution. If such was the case, the increased relative injury as compared with the solution cultures must have been due to the precipitated, but probably slightly soluble, ferric phosphate and hydroxide.

The results given in tables 2 and 3 and plotted in figures 2 and 3 seem to indicate that in solution "A" the toxicity of iron and aluminum salts is largely due to the acidity produced when the salts are precipitated. The relatively small effect of large amounts of iron and aluminum salts when added to this solution confirms the well known power of calcium- and phosphoric-acid soil amendments to precipitate and render harmless any heavy metals which may be present in the soil.

The relative amount of transpiration is in most cases closely related to the yield. In the solution cultures, there seems to be a tendency for the transpiration to be depressed more than the yield.

No important specific pathological conditions were observed in any of the acid cultures. In plants grown in the solution cultures containing toxic concentrations of iron salts, zone "B" of the lower nodes was invariably discolored brown or reddish brown. The number of discolored nodes varied. In some cases, almost every node was discolored: the upper ones almost a rose color, the lower much darker. In no other cultures did discolorations of this type appear. In the sand cultures the results were very similar except for the lesser discoloration by the ferric salts. A 0.0002 *N* ferrous sulphate solution caused a reddish-brown discoloration of all the nodes from which roots arose in four of the ten plants. The 0.0004 *N* solution discolored the lower nodes in all the plants and some of the nodes above the roots in three. It is very likely that if the plants had been grown for a longer period, these cultures would not have produced relatively as good a growth in comparison with the control. The 0.0004 *N* ferric solutions did not produce any discoloration. The nodes, however, did show a strong accumulation of iron in the node when haematoxylin was applied after the method of Moore ('14). In some of the more toxic concentrations of the acids and of the aluminum salts, plants were found, occasionally, in which the lowest nodes were somewhat yellow or a yellowish brown. Pieces of these nodes were placed on agar slants by Dr. Alice M. Russell. In most instances yellow colonies of bacilli developed on the slants. Dr. Russell believes that these organisms were largely responsible for the yellow discoloration. Pieces from the nodes discolored by iron were also placed on agar by Dr. Russell. Organisms were secured in only a very few instances. This fact, and the absence of a reddish-brown discoloration in all solutions not containing iron in toxic concentrations, seem conclusive proof that the iron salts are the primary cause of this pathological condition. Michrochemical tests made by the method developed by Carr[2] on the plants grown in the solutions containing the aluminum salts, showed that aluminum collected in zone "B" similarly to the iron salts but produced no discoloration. Chlorotic plants were commonly found in the cultures containing toxic concentrations of aluminum salts. The nitrate usually produced the most severe chlorosis.

---

[2] The test used was developed by Dr. R. H. Carr of the Office of Cereal Investigations, U. S. Department of Agriculture. The distribution of iron was determined microchemically in one half of the stem by an acidulated solution of KCNS. The other piece was then boiled in a saturated solution of $(NH_4)_2CO_3$ to which a small quantity of haematoxylin was added. Carr's analyses have shown that any increased discoloration produced by the second test indicates the presence of aluminum.

The author does not wish to indicate that aluminum or iron salts are the only ones which may produce a pathological condition in zone "B." These results are to be applied only to the variety of yellow dent field corn which was used in this investigation. It has been pointed out by True ('21) that the metabolism of sweet corn is very different from that of field corn.

The results of the present investigation indicate that from both an agricultural and an ecological viewpoint it is important to know both the H-ion concentration and the composition of the soil. Field corn is evidently very tolerant of a fairly high acidity if no aluminum or iron salts are present. It is very likely that under field conditions the salts of these metals are more toxic than the results of this investigation indicate because of their unfavorable effect upon root development. Injury by iron salts can be determined readily by the color of the nodes. Microchemical tests will be necessary to determine the presence of aluminum.

SUMMARY

Yellow dent field corn was grown in two nutrient solutions of a greatly different composition to determine the effect of the composition of the nutrient solution; first, upon the amount and form of iron necessary for optimum growth; second, upon the toxicity of sulphuric, nitric, and hydrochloric acids and their corresponding salts with iron and aluminum. The solutions had the following composition: Solution "H," $CaH_4(PO_4)_2$, 0.00005 *M;* $Ca(NO_3)_2$, 0.0015 *M;* NH_4NO_3, 0.001 *M;* KCl, 0.0008 *M;* $MgSO_4$, 0.0008 *M;* $Al_2(SO_4)_3$, 0.000003 *M;* $MnSO_4$, 0.00001 *M;* $ZnSO_4$, 0.000005 *M;* Solution "A," KH_2PO_4, 0.0024 *M;* $Ca(NO_3)_2$, 0.0036 *M;* $MgSO_4$, 0.0035 *M.* Sand cultures were used to check the results obtained in the solution cultures with solution "H."

The availability of the iron in ferric phosphate for the growth of corn was found to depend largely upon the composition of the nutrient solution. A concentration of 7 mg. per liter was sufficient for the optimum growth in solution "H." Plants grown in solution "A" with five times this amount were unable to secure sufficient iron.

The optimum growth in solution "A" was secured with 0.0005 *N* ferrous sulphate. The addition of ferric nitrate to this solution produced a precipitate from which the plant was unable to secure sufficient iron even with a 0.001 *N* concentration. The plants in this concentration as well as in one of 0.002 *N*, which was strongly toxic, were chlorotic.

Sulphuric, nitric, and hydrochloric acids were about alike toxic when they were added to either solution in low concentration. At the highest concentration used, sulphuric acid depressed the growth of the tops less

than the same normality of the other two acids. This difference was even more favorable to the sulphuric acid when the root development was compared. The nitrates and chlorides of aluminum and iron also depressed root growth more than the sulphates.

With solutions "A" and "H," an initial H-ion concentration less than pH 3.7 had little effect upon the rate of growth. In most cases the plant tended to shift the reaction toward neutrality. On the contrary, when ferrous sulphate and certain concentrations of the chlorides were added to the cultures, plant growth increased the initial H-ion concentration of the solution. The reaction was most readily shifted toward neutrality when the acidity was due largely to sulphuric acid and least readily when due to hydrochloric acid. The amount of shifting of the reaction was approximately the inverse of the toxicity of the salt or of the acid; *i.e.*, the change in reaction was proportional to the size and the activity of the plant.

The toxicity of ferrous sulphate showed no relation to the initial H-ion concentration. A 0.0002 *N* concentration depressed growth 27 percent in solution "H." The ferrous sulphate was approximately twice as toxic as the same normality of the ferric salts. The latter were more readily precipitated, and their depressing effect was closely related to the H-ion concentration produced by their hydrolysis or precipitation.

In solution "A" the aluminum salts were readily precipitated, and their toxicity was evidently due largely to the H-ion concentration thus produced. In solution "H" the toxicity was due to the aluminum ion. A 0.0002 *N* concentration produced about the same depression as the same normality of ferrous sulphate. The development of the secondary roots was inhibited by a 0.0006 *N* concentration. The nitrate seemed to be somewhat more toxic than the other aluminum salts and showed a greater tendency to produce chlorosis.

Sand cultures greatly increased the concentration of acids necessary to produce the same effect when compared to their effect in the solution cultures. The same concentration of the salts produced about the same depression of the relative weights of the tops in both types of cultures. The use of the sand medium lowered the toxicity of the salts to the roots proportionally more than it increased the yield of the tops.

The composition of the nutrient solution had a greater influence in determining the toxicity of the salts than the medium in which the plants were grown.

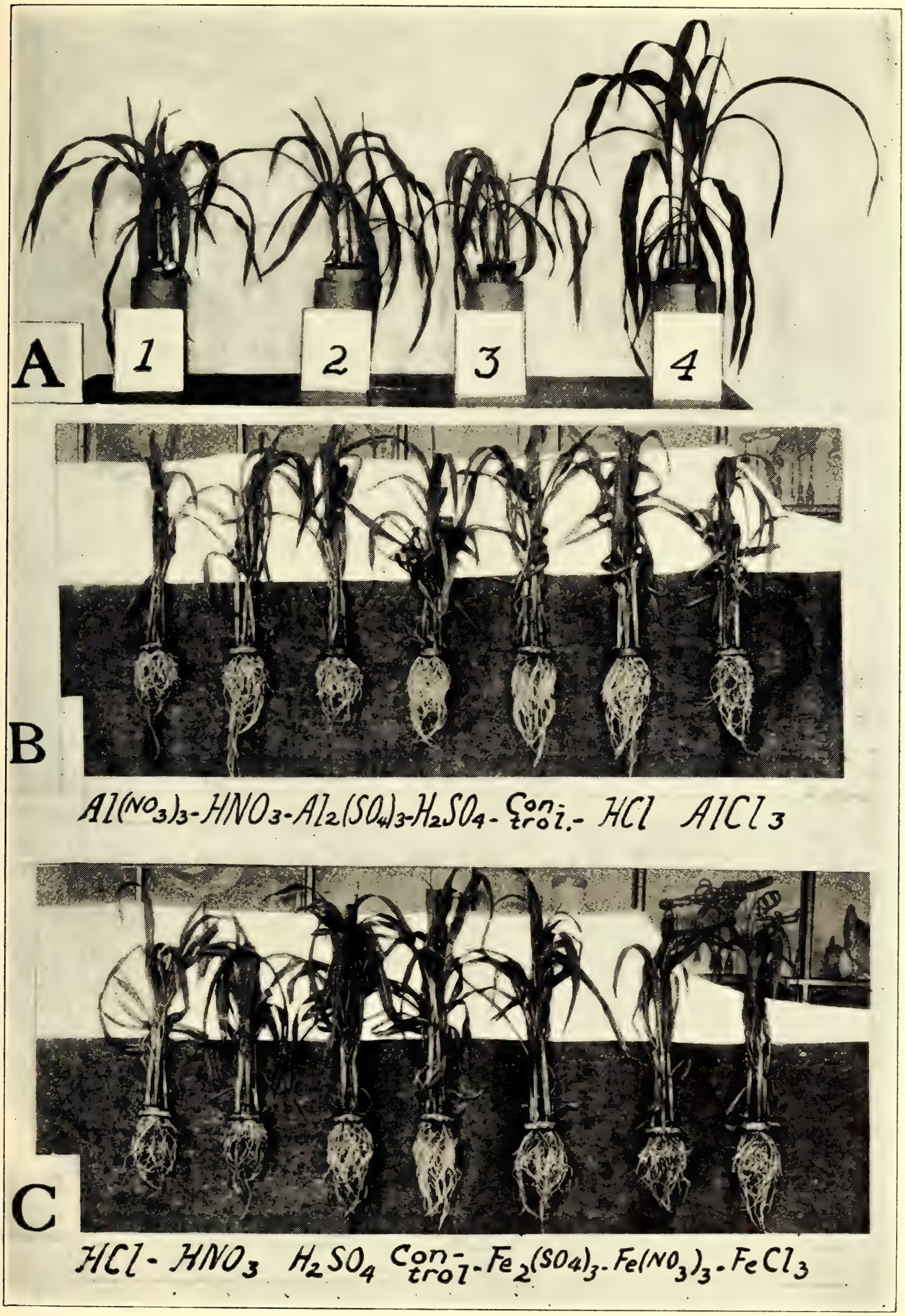
Iron salts when present in injurious concentrations produced a reddish-brown discoloration of the lower portion of the nodal area. Aluminum salts collected in the same position but produceed no discoloration.

Botanical Laboratories,
University of Pennsylvania

BIBLIOGRAPHY

Abbott, J. B., Conner, S. B., and Smalley, H. R. ('13). Ind. (Purdue Univ.) Agr. Exp. Sta. Bull. 170. 1913.

Awatsu, H. ('14). Arch. Int. Pharm. Therap. 23: 325–352. 1914.


A
1
2
3
4
B
$Al(NO_3)_3$ - HNO_3 - $Al_2(SO_4)_3$ - H_2SO_4 - Control. - HCl $AlCl_3$
C
HCl - HNO_3 H_2SO_4 Control - $Fe_2(SO_4)_3$ - $Fe(NO_3)_3$ - $FeCl_3$

ARNDT: GROWTH OF FIELD CORN

Berthelot and André, G. ('95). Compt. Rend. Acad. Sci. Paris **120**: 288–290. 1895.
Boiret, H., and Paturel, G. ('92). Ann. Agron. **18**: 417–440. 1892.
Bordnár, J. ('15). Biochem. Zeitschr. **69**: 245–256. 1915.
Clark, W. M. ('20). The determination of hydrogen ions. Baltimore, 1920.
Conner, S. D. ('21). Jour. Amer. Soc. Agron. **13**: 113–124. 1921.
Corson, G. E., and Bakke, A. L. ('17). Proc. Iowa Acad. Sci. **24**: 477–482. 1917.
Czadek, O. von ('04). Zeitschr. Landw. Versch. Oesterr. **7**: 65–67. 1904. Abstr. in Exp. Sta. Record **15**: 964. 1904.
Daikuhara, G. ('14). Bull. Imp. Cent. Agr. Exp. Sta. Japan **2**: 1–40. 1914.
Duggar, B. M. ('11). Plant physiology. New York, 1911.
—— ('20). Ann. Mo. Bot. Gard. **7**: 1–49. 1920.
Free, E. E. ('15). Plant World **18**: 249–256. 1915.
Gile, P. L., and Carrero, J. O. ('20). Jour. Agr. Res. **20**: 32–62. 1920.
Hartwell, B. L., and Pember, F. R. ('07). Ann. Rept. R. I. Agr. Exp. Sta. **20**: 358–380. 1907.
—— ('08). Ann. Rept. R. I. Agr. Exp. Sta. **21**: 286–294. 1908.
—— ('18). Soil Sci. **6**: 259–277. 1918.
Hoagland, D. R. ('17). Soil Sci. **3**: 547–560. 1917.
—— ('19). Jour. Agr. Res. **18**: 73–117. 1919.
Hoffer, G. N., and Carr, R. H. ('20). Phytopath. **10**: 56, 57. 1920.
Joffe, J. S. ('20). Soil Sci. **9**: 261–266. 1920.
Johnson, E. S. ('20). Soil Sci. **10**: 389–409. 1920.
Katayama, T. ('06). Bull. Coll. Agr. Tokyo **7**: 91–93. 1906.
McCall, A. G. ('16). Soil Sci. **2**: 207–253. 1916.
Maquenné, L., and Demousy, E. ('20). Compt. Rend. Acad. Sci. Paris **117**: 218–222. 1920.
Mazé, P. ('13). Ann. Inst. Pasteur **27**: 651–681. 1913.
—— ('15). Compt. Rend. Acad. Sci. Paris **160**: 211–214. 1915.
—— ('19). Ann. Inst. Pasteur **33**: 139–173. 1919.
Meurer, R. ('08). Jahrb. Wiss. Bot. **46**: 502–567. 1908.
Mirasol, J. ('20). Soil Sci. **10**: 153–218. 1920.
Miyake, J. ('16). Jour. Biol. Chem. **25**: 23–26. 1916.
Moore, B. ('14). Proc. Roy. Soc. London, B, **87**: 556–570. 1914.
Noyes, H. A. ('19). Jour. Ind. Eng. Chem. **11**: 1040–1042. 1919.
Rothert, W. ('06). Bot. Zeit. **64**: 43–52. 1906.
Rupprecht, R. W. ('15). Mass. Agr. Exp. Sta. Bull. 161. 1915.
Salter, R. M., and McIlvaine, T. C. ('20). Jour. Agr. Res. **19**: 73–95. 1920.
Shive, J. W. ('15). Physiol. Res. **1**: 357–378. 1915.
—— ('20). Jour. Agr. Res. **18**: 327–397. 1920.
—— **and Jones, L. H.** ('21). Soil Sci. **11**: 93–100. 1921.
Stoklasa, J., et al. ('18 *a*). Biochem. Zeitschr. **88**: 292–322. 1918.
—— ('18 *b*). Biochem. Zeitschr. **91**: 137–232. 1918.
Sturtevant, E. L. ('94). Bull. Torrey Bot. Club **21**: 319–343. 1894.
Szücs, J. ('12). Jahrb. Wiss. Bot. **52**: 269–332. 1912.
Tottingham, W. E. ('14). Physiol. Res. **1**: 133–245. 1914.
—— **and Hart, E. B.** ('15). Jour. Agr. Res. **5**: 233–250. 1915.
True, R. H. ('21). Jour. Amer. Soc. Agron. **13**: 91–107. 1921.

EXPLANATION OF PLATE IV

FIG. *A*. Relative growth in solutions "A" and "H" when $FePO_4$ was supplied as a source of iron. Numbers 1, 2, and 3 are in solution "A," with 3.5, 14, and 35 mg. $FePO_4$ per l., respectively. Number 4 is solution "H" with 7 mg. $FePO_4$ per l.

FIG. *B*. Effect of 0.0004 *N* acids and their aluminum salts in solution "H."

FIG. *C*. Effect of 0.0004 *N* acids and their ferric salts upon root development in solution "H."

CONTROL OF THE SEXUAL STATE IN ARISAEMA TRIPHYLLUM AND ARISAEMA DRACONTIUM [1]

John H. Schaffner

(Received for publication June 2, 1921)

Arisaema triphyllum (L.) Torr., being a dioecious plant with a considerable percentage of intermediate individuals, presented itself to the writer's mind as a very favorable plant for experiments on the control of sex, such experimental control seeming to be necessary to substantiate conclusions reached previously, mainly from taxonomic studies, as to the fundamental nature of the sexual state. Accordingly, work was begun on this plant in the spring of 1917. More recently work has also been done on *Arisaema Dracontium* (L.) Schott.

The first plantings were made in pots and were not very satisfactory, probably because there was no place in the greenhouse to keep them properly, and the writer was absent during the entire winter. However, one pure carpellate plant changed to a pure staminate plant, and some intermediate individuals also changed to the staminate condition. The writer was certain, therefore, that sex reversal took place, and the results so far as they went agreed with the reports of sex reversal in Arisaema by Atkinson (1), Gow (2), and Pickett (3). The study was continued by observations in the field and by experiments in garden plots.

Field Observations

The individuals in the field are pure staminate, pure carpellate, or intermediate in varying degrees from nearly pure staminate or carpellate to individuals having inflorescences with about an equal number of staminate and carpellate flowers. There are usually about 60 to 80 flowers on a fairly large inflorescence. The folding of the margins of the spathes appears to be an ordinary fluctuation. There are about equal numbers folded clockwise and contra-clockwise. The same conditions hold for the spathes of *Arisaema Dracontium.*

As stated, the intermediate plants show all degrees of sexual expression. The inflorescence may be carpellate except for one imperfect or perfect staminate flower, or it may be staminate with a single carpellate flower, or from such a condition up through all gradations to a monoecious inflorescence with about an equal number of both kinds of flowers. There is no definite position for the appearance of the opposite type of flowers. Pickett (3) has reported similar conditions. The staminate and carpellate areas are sometimes in definite spots, and when the transition line happens to pass through a flower one side is staminate and the other carpellate. The writer has published a detailed account of such cases elsewhere (6).

---

[1] Papers from the Department of Botany, The Ohio State University, no. 130.

As a general rule the carpellate plants are the larger. There is an important difference in the longevity of the peduncle. The staminate peduncles die and dry up soon after blooming, while the fertilized carpellate peduncles, of course, remain in a vigorous condition until the fruit is matured. In the intermediate plants, there is every gradation of robustness and persistence, depending on the degree of carpellateness of the individual. Those that are largely staminate, though they may have several well-developed green fruits, soon wither, while those that have but a few staminate flowers remain green and erect. There is no essential difference in the vegetative characters of the staminate and carpellate individuals. Both one-leaved and two-leaved plants are either staminate or carpellate in about equal proportions.

A statistical study of the plants in the field was made with the results shown in table 1, all plants showing any evidence of monoeciousness being classed as intermediate.

TABLE 1. *Arisaema triphyllum in the Field*

Habitat	Carpellate Plants		Intermediate Plants		Staminate Plants	
	Number	Percentage	Number	Percentage	Number	Percentage
I. Rich beech forest......	315	26+	167	13+	727	60+
II. Open pastured wood...	43	13+	40	12+	227	73+
III. Rich, moist mixed forest	9	23+	7	17+	23	59+
IV. Base of north-facing limestone cliff in ravine, rather dry....	16	10+	15	10	119	79+
V. Rich mixed forest containing black humus .	61	36+	38	22+	67	40+
Totals and averages for the 1874 plants..............	444	23+	267	14+	1163	62+

It will be seen by an inspection of the table that there is a large fluctuation in sex ratios for the different habitats. The staminate plants are always much in excess of either the carpellate or the intermediate individuals, and with the exception of the wet black humus habitat considerably over 50 percent of the total. The fourth habitat, representing a rather dry, poor soil, is in striking contrast to the fifth and shows how plants are affected by the ordinary field environments. The rich, wet soil has, in proportion, more than three times as many carpellate plants as the poor, dry soil, more than twice as many intermediates, and only half as many staminate individuals. As is shown below, these extreme fluctuations are due to the direct control of the sexual state by environmental factors and not to a difference in death rate between the two sexes.

EXPERIMENTAL PLOTS

In order to determine whether the sex could be reversed under definite control, a small number of plants was dug up on June 14, 1919, and transplanted into a special bed on the north side of the greenhouse at the west end. There were 25 pure carpellate plants, 5 intermediate plants, and 10 pure staminate plants. They were all treated alike, having most of their roots removed and all but a small fragment of the leaf blade cut away. The habitat was rather dry, and no artificial watering was done after the first few days. The date of transplanting was rather late, and it was feared that the incipient inflorescence buds might already have their sexual state determined. This fear was, however, mostly unfounded, as the next spring's crop of flowers proved. Under the treatment all the plants were expected either to stay in the pure male state or to change over to the male condition.

The following was the result in the spring of 1920:

Of the 10 originally staminate plants, one died and 9 were still pure staminate.

All of the 5 originally intermediate or monoecious plants were pure staminate.

Of the 25 originally pure carpellate plants, 21 changed to pure staminate, 2 remained pure carpellate, and 2 had intermediate inflorescences. One of the originally carpellate plants gave rise to pure staminate "vegetative twins" which were counted as a single plant in the statistics. Of the two intermediate individuals, one had a carpellate inflorescence with two staminate flowers at the top and the other had a carpellate inflorescence with seven staminate flowers at the top.

Under the treatment, therefore, all the monoecious plants changed to the male condition, and out of the 25 carpellate plants 21 were reversed from a pure female condition to a pure male condition, 2 were changed from a pure female condition to a hermaphroditic condition, and 2 were either not influenced or else their buds had already developed the flower incepts beyond the stage at which reversal is possible.

The inflorescences were all removed in 1920 as soon as they were out of the ground, and all three plots were then treated with a thick layer of well rotted cow manure and kept very wet until the end of June. The plants made an extraordinary growth, and the intention was to reverse the sex in the opposite direction in all three plots, *i.e.*, from staminate to carpellate, the presumption being that high nutrition or abundant water supply or both combined would produce such a physiological condition in the corms that the incipient inflorescence buds would be thrown into the female state. The results in the spring of 1921 were as follows:

The 9 plants which were originally staminate and which had remained staminate the previous season were all reversed and carpellate, the female condition being complete in all except one individual which had three staminate flowers at the tip of the inflorescence.

Of the 5 plants which were originally intermediate or monoecious, and which had been changed to the pure staminate condition the previous season, 4 were pure carpellate and one which had given off a large branch the first year and whose corm was thus reduced gave rise to 2 staminate shoots.

Of the 25 plants which were originally pure carpellate, and of which 23 had been caused to change their sexual state the previous season, one failed to bloom, one was pure staminate, one was intermediate with a carpellate inflorescence showing a single staminate flower at the top, and 22 were pure carpellate, having reversed their sexual state back to the condition they were in when originally transplanted.

The corms have given off a considerable number of small buds, so it is probable that future reversals will not be so nearly universal as in the first two seasons. The twin plant of the previous season had separated its corms completely, and both the twins that had been completely staminate the previous season were now completely carpellate.

The experiments show conclusively that it is possible to exercise almost complete control over the sexual state of *Arisaema triphyllum*. A slight disturbing factor is introduced because of the fact that the corms often produce buds of a sufficient size to rob the parent of much of its food supply. Sex reversal is complete in the individual in either direction from time to time, the male to the female or *vice versa*, or the reversal may be partial, from a pure male or female state to a hermaphroditic condition, or *vice versa* from a hermaphroditic condition to a pure male or female state. The exact factor which induces the reversal has not been ascertained. The writer was desirous to show first of all that sex reversal actually takes place in many plants. Apparently in *Arisaema triphyllum* the sexual state depends mainly on the water supply, or on the nutrition, or on both combined. According to Atkinson's (1) experiments the determining factor might be the varying amounts of the nutriment in the corm. Pickett (3) believes his field observations and experiments show that "the amount of water available at a certain period in development is directly or indirectly responsible" for the given sexual state. Apparently the sexual state may be determined in a feeble or in an intense manner. If the female state is of low degree, the inflorescence may change from carpellate below to staminate toward the top, or in some cases any patch of cells may change to the male state whatever their position in the spadix. If the sexual state is originally determined in the bud as male, the reversal usually takes place in patches at the sides of the spadix, involving one or more flowers, apparently without any relation to the age of the cells or to their position on the inflorescence axis.

Arisaema triphyllum is an unusually favorable perennial herb for experimental purposes in studies on sex control and reversal, and there should be no special difficulty in determining the exact environmental factor or group of factors which determine maleness or femaleness in the incipient inflorescence bud.

ARISAEMA DRACONTIUM

Some work has been done on *Arisaema Dracontium* both experimentally and in the field, although it is much less common around Columbus than *A. triphyllum*. In this species, so far as the writer's knowledge goes, there are staminate and monoecious individuals. No pure carpellate plants have been discovered, although there are decided differences in the relative widths of the carpellate and staminate zone between different monoecious individuals. One plant was found that came very near to a pure carpellate inflorescence. The normal part of the flower-bearing spadix was purely carpellate, but on the sterile part above this there were six scattered staminate and imperfectly staminate flowers. One of these flowers was half carpellate. It is probable that in rare instances pure carpellate inflorescences are produced. One "staminate intermediate" plant was found having three carpellate flowers on one side at the base of the inflorescence, which was otherwise normally staminate; another staminate plant had two carpellate flowers near the middle of the spadix on opposite sides. Such intermediate staminate plants, even though they have the abnormal carpellate flowers fertilized, soon wither like ordinary staminate peduncles as in similar cases in *A. triphyllum*. Abnormal monoecious individuals were also found. One of these had two staminate flowers at the base, then a zone about one fourth inch wide of carpellate flowers, and above this a zone three fourths inch wide of staminate flowers; another had a single carpellate flower in the middle of the staminate zone; another was nearly completely staminate at the base, but in this there were three carpellate flowers, next there was a complete narrow zone of carpellate flowers, and above a wide zone of staminate flowers again. Three other monoecious inflorescences had each several staminate flowers at the base of the carpellate zone. One monoecious plant had the lower part in irregular staminate and carpellate patches. In those inflorescences which have staminate and carpellate flowers in patches, as well as in the ordinary monoecious type, there is often a general confusion of floral structures, as reported by the writer (6) for *A. triphyllum*. There are flowers partly staminate and partly carpellate, staminate flowers whose stamens have stigmas, and neutral or partly neutral structures of various abnormal shapes. These examples show that, as in *A. triphyllum*, there are intermediate individuals which do not have the ordinary types of sexual expression.

A statistical study of two habitats for two years gave the results shown in table 2.

The plants in the second habitat (III and IV) are apparently dying out rapidly because of changing ecological conditions and the ravages of the Arisaema rust. In the first habitat the plants are thriving. In both habitats and in both years there is an enormously greater number of staminate individuals than of monoecious individuals.

TABLE 2. *Arisaema Dracontium in the Field*

Habitat	Monoecious Plants		Staminate Plants	
	Number	Percentage	Number	Percentage
I. Rich, moist, open mixed wood, 1920 .	9	8+	97	91+
II. The same in 1921....................	125	18+	568	81+
III. Open pastured wood, 1920..........	2	4+	43	95+
IV. The same in 1921....................	1	3+	27	96+

On May 28, 1920, a plot on the north side of the greenhouse was planted with several monoecious individuals and a considerable number of staminate individuals, the leaf surface and the roots being reduced. The intention was to give the plants an environment that would keep the staminate plants staminate and change the monoecious individuals to staminate individuals. In the spring of 1921, three of the originally monoecious plants bloomed; two were monoecious and one was pure staminate. Seventeen of the originally staminate plants bore inflorescences, of which sixteen were pure staminate and one typically monoecious. This monoecious plant had changed from the staminate condition in spite of the fact that the nutritive supply was decreased. Presumably, its bud for the following year's shoot was already sufficiently developed to have its sex determined, or else it had a sufficient amount of the proper food supply stored in the corm to cause a reversal of sex notwithstanding the unfavorable treatment given.

But the important result is that in *A. Dracontium* staminate individuals do reverse to monoecious individuals, and that monoecious individuals do change to staminate individuals. The conditions of sexuality are in general the same as in Jack-in-the-pulpit. Sex in the green-dragon is a condition and not dependent on homozygous and heterozygous hereditary factors. The general hereditary constitution is apparently such that when the female state is established in the incept of the monoecious inflorescence, the condition is not strong enough to continue through the entire developmental cycle; but after the bud has developed a zone of carpellate flowers, the sexual state is reversed, perhaps through senile changes of the cells, specific differentiations, or exhaustion of available food supply, and only staminate flowers are produced above. *Arisaema Dracontium* is, therefore, a species with decidedly male characteristics and can be compared with a similar condition in the gametophyte generation of *Selaginella Kraussiana* and other like species where, because of the small percentage of megasporophylls and megaspores produced, the female gametophytes are outnumbered to an extreme extent by the males; or it may be compared with such animals as the European cuckoo bird, which is said to produce about four males or even more to one female.

Conclusions

The foregoing study shows that sex in Arisaema is dependent on a functional state and not on hereditary factors; that the sexual state is readily controllable and is reversible in either direction, the male to the female or the female to the male, and then back again; and that the dimorphism which appears in the inflorescences of these diploid organisms cannot be due to homozygous and heterozygous factors or chromosome constitutions.

The condition of affairs found in Arisaema is in perfect agreement with that discovered by the writer in *Cannabis sativa* (5) and by Yampolsky in *Mercurialis annua* (7), which are very different types of plants and belong to different subclasses of a different class. The factorial hypothesis of sex cannot be entertained by botanists, and it is the writer's conviction that sex in the higher animals is no more determined by Mendelian factors than in plants and that chromosome differences where they exist are merely indicators of sex and not determiners. Chromosome differences may influence the metabolism of the cell and thus influence the determination of sex, but it is possible to overcome this influence in the cell and to cause a reversal of the sexual state even in the presence of an allosome difference. In fact, the sexual state is commonly reversed in the somatic cells of animals through various external causes, as by the injection of proper hormones, removal of sex glands, etc.

In nature, we see quite generally the existence of positive, negative, and neutral conditions, and the physicist is inclined to interpret these conditions in terms of positive and negative electricity. Whatever the fundamental cause of the positive and negative states of matter will be found to be, it will probably also give the clue to the nature and cause of the remarkable duality and dimorphism which we call sexuality and which is a characteristic of all plants and animals except the very lowest.

LITERATURE CITED

1. **Atkinson, G. F.** Experiments on the morphology of *Arisaema triphyllum* [Abstract]. Bot. Gaz. **25**: 114. 1898.
2. **Gow, J. E.** Observations on the morphology of the Aroids. Bot. Gaz. **56**: 127–142. 1913.
3. **Pickett, F. L.** A contribution to our knowledge of *Arisaema triphyllum*. Mem. Torrey Bot. Club **16**: 1–55. 1915.
4. **Schaffner, J. H.** The expression of sexual dimorphism in heterosporous sporophytes. Ohio Jour. Sci. **18**: 101–125. 1918.
5. ——. Influence of environment on sexual expression in hemp. Bot. Gaz. **71**: 197–219. 1921.
6. ——. Reversal of the sexual state in certain types of monoecious inflorescences. Ohio Jour. Sci. **21**: 185–200. 1921.
7. **Yampolsky, C.** Inheritance of sex in *Mercurialis annua*. Amer. Jour. Bot. **6**: 410–442. 1919.

Columbus, Ohio

A DEMONSTRATION OF NUMEROUS DISTINCT STRAINS WITHIN THE NOMINAL SPECIES *PESTALOZZIA GUEPINI* DESM.[1]

Carl D. La Rue and H. H. Bartlett

(Received for publication June 6, 1921)

In the course of routine pathological work on the plantations of the United States Rubber Company, in Asahan, East Coast of Sumatra, we found that Pestalozzia was among the fungi most often isolated from diseased tissues of *Hevea brasiliensis*. A comparison of several of the strains obtained showed that all were very similar as far as obvious characters were concerned, and all fell within the limits of *Pestalozzia Guepini* Desm., as this species has been broadly interpreted; but they were quantitatively different from one another, and maintained their distinctness through successive generations. Since none of the diseases of rubber which have been attributed to Pestalozzia are particularly important, and since the time at our disposal was limited, only enough measurements were made to demonstrate that the nominal species in this group of fungi may be looked upon as composed of a large number of pure lines, each definable by its mode and range of variation. Looked upon in this light, the Fungi Imperfecti doubtless afford a parallel to the host of constant but hardly perceptibly distinct strains in some groups of the higher plants, such as Hieracium, in which the constancy of the lines is maintained because of the loss of sexual reproduction. The problem of species delimitation in either case is a most difficult one. Among many fungi the close restriction of strains to special hosts gives criteria for the separation of species which have never been differentiated morphologically, although in many such cases morphological as well as physiological distinctions might often be established if more refined biometrical methods were used than those which prevail in systematic work. The truth of this statement, as well as the great advances in mycology which may be expected to follow the more general introduction of biometrical methods, are illustrated by the notable studies of Stakman and Levine (1) in the rusts, and of Rosenbaum (2) in Phytophthora.

Only morphological criteria, however, are available to the systematist in those weakly parasitic genera whose members are not confined to specific, or even closely related, hosts. This fact is coming more and more to be recognized, and mycologists nowadays much less often than formerly attempt to clothe superficial systematic work in the garb of respectability by assuming host delimitation for forms belonging to groups in which no clear case for such delimitation has been made out. Morphologically indistinguishable fungi not known to be confined to particular hosts are best called by the same name. It is idle, for example, to use one name for the Pestalozzia strains causing the gray blight of tea and another for those which cause leaf spot of palms. In both cases, as we have found, the strains show differences among themselves, covering a wide range of variation, just as do the strains from Hevea. Our isolations from hosts other than Para rubber were made in order to find out whether or not the strains infecting these hosts were distinct, and, providing they were, whether or not they had served as sources of infection for Hevea.

---

[1] From the Botanical Laboratory of the Hollandsch-Amerikaansche Plantage Maatschappij, Kisaran, Asahan, Sumatra. Published by permission of the United States Rubber Plantations, Inc.

Of all the common hosts of Pestalozzia in the East Indies, it seemed least likely that Hevea would be found to be infected with specifically restricted strains. In fact, it appears that all the common diseases of Para rubber in the Orient are due to more or less ubiquitous fungi, and that no serious, specific diseases of Hevea were introduced from Brazil with the original rubber seed. It is accepted by Burkill (3), who has gone over the existing records with care, that all the oriental Hevea has descended from a single introduction of seeds collected for the Government of British India on the upland plateaus near the valley of the Tapajos River in Brazil. The seeds were grown at Kew, the latter institution having served the purpose of the modern quarantine station, for no diseased stock seemed to have been forwarded to Ceylon and Malaya. In consequence, the causes of rubber diseases in the East are found among those fungi which are little restricted as to host. Generally speaking, the forms pathogenic to Hevea have been reported as causing diseases of the most diverse cultivated plants.

In view of these facts it was natural that we should attempt to identify the Pestalozzia strains of Hevea with those of other plants from which these fungi have been described or reported. As with so many other genera of Fungi Imperfecti, the descriptions of the nominal species of Pestalozzia are generally in no degree diagnostic. There are whole groups of descriptions of which nothing whatever is left, when common factors are canceled, except the names of different host plants and different geographic localities. Quantitative data, when given, are seldom, if ever, stated so that one is convinced of their accuracy.

Pestalozzia is chiefly known in the eastern tropics through causing the gray blight of tea and the leaf spot of the cocoanut palm. The literature shows that several specific names have been applied to the causal fungi with only the vaguest notions of specific delimitations in the group. Of these names the oldest is *Pestalozzia Guepini* Desm. (first published by Desmazières (4) as *Pestalotia Guepini*). It was originally applied to a form or forms from Camellia and Magnolia, characterized by a spore length of about one fiftieth millimeter, with four-septate spores, the terminal and basal cells hyaline, and the three interior cells dark; appendages longer than the spores and typically three in number.

More recently, diseases of several tropical cultivated plants, including the tea blight and a stem disease of rubber seedlings, have been attributed to *Pestalozzia palmarum* Cooke. This name was originally based by Cooke in 1876 (5) upon a fungus from the dead sprout of a cocoanut from Bengal. The spores were stated to be 0.015 mm. long. Later, in 1877, the same author (6) supplemented the description from material found on the dead stems of *Cocos nucifera* from Demerara, giving the length of the colored portion of the spores (*i.e.*, the three inner cells) as 0.045 mm. Cooke was so notoriously careless that it is useless to try to explain the discrepancy in the measurements, or to speculate as to whether or not the material from the two sources was the same. Discarding the measurements as hopelessly irreconcilable, the description affords no distinction from *Pestalozzia Guepini* Desm., except the different host.

In 1872, before Cooke had named *Pestalozzia palmarum* at all, he had described the causal fungus of the gray blight of tea as *Hendersonia theaecola* (7). There is no doubt that this was merely Pestalozzia without the terminal and basal hyaline cells, which fall off in old, weathered specimens. Cooke himself suggested that such was the case, comparing his so-called Hendersonia, in the original description, with *Pestalozzia Guepini*. Recently the tea blight has been christened again by Sawada (8) as *Pestalozzia Theae*. Sawada is hardly to be blamed for neglecting Cooke's defective description and incorrectly formed name, but unfortunately he fails to make out a clear case for the distinctness of the tea fungus. His description (as translated by Tanaka, in Mycologia) gives the length of the spores as 16 to 21 μ, but in a letter dated June 26, 1919, he gives 23 to 31 μ; the latter measurement is doubtless the correct one. Sawada's idea of *Pestalozzia Guepini* is that of a form differing from the tea-blight fungus in having shorter and generally three-septate rather than four-septate spores. Desmazières' description, however, makes it very clear that in the original material of *P. Guepini* the spores were typically four-septate, and therefore five-celled, the three central cells being dark in color. Moreover, his measurement of the length, about one fiftieth millimeter, although vague, appears to be quite as dependable as the measurements of later authors, and would not exclude from *P. Guepini* any of the tropical Pestalozzias which have been isolated from tea, cocoanut, rubber, etc. Sawada states that in *P. Theae* the appendages are club-shaped, whereas in *P. palmarum*, which he records as found upon tea in Formosa, they are filiform. This character is one upon which it seems unwise to place reliance, since any strain may show both conditions, depending upon circumstances. In the last analysis it is the only character used by Sawada for the differentiation of two supposedly distinct fungi found upon the same host in the same locality, and we are unable to grant its validity. The literature shows, as far as tropical diseases caused by Pestalozzia are concerned, that authors have, in general, used the names *P. Guepini* and *P. palmarum* interchangeably, perhaps preferring the former name for tea diseases and the latter for palm diseases, but no one has justified the latter usage either by cross-inoculation studies or by finding dependable morphological distinctions.

We were unable to attempt cross-inoculation studies in the time at our disposal. Likewise, as advocates of biometrical methods in systematic work, we feel called upon to apologize for the scantiness of our statistical data, which were obtained as a by-product, as it were, of other work. We were in general unable to make more than twenty-five measurements of spore length, and an equal number of appendage length, in each generation. However, we have data for each strain covering from four to eight generations. The material proved unusually favorable for statistical study because of the presence of two independent and easily measured variables, namely, spore length and appendage length. In the measurement of spore appendages we chose at random one of the three appendages of each spore, by the simple expedient of measuring whichever appendage lay most perfectly in the focal plane.

In spite of the fact that our procedure might have been improved, we feel that our general conclusion, that the nominal species in the Fungi Imperfecti can be resolved into a large number of quantitatively distinct strains, is well established. Greater refinement of method would merely have increased the significance of the differences between the more closely similar groups of strains, and could only have confirmed the conclusion that might have been drawn from purely theoretical considerations, namely, that in the Fungi Imperfecti, a group of non-sexual organisms, every hereditary modification, of whatever magnitude, must give rise to a pure line. These pure lines are too numerous and too incapable of precise recognition to be named. Consequently the concept of species must be arbitrary and ruled by practical considerations, the species including a large group of distinct strains. Any one strain is capable of precise definition within certain limits, established by the degree of refinement of environmental control and biometrical accuracy, but within these limits it is indistinguishable from other strains which theoretically may exist, but cannot be recognized.

Our cultures were all grown upon Hevea-leaf agar with native brown sugar, the latter being the boiled-down sap of the sugar palm, *Arenga saccharifera*. Different lots of medium were fairly uniform. On the whole, the successive generations were subjected to no more environmental fluctuation than would probably obtain in the average laboratory, since temperature conditions in Asahan are very uniform. No effort was made to keep the cultures at constant temperature. The strains showed physiological differences among themselves, indicated by the different lengths of time required for the production of spores and by the varying abundance of spores produced, but the differences were not correlated with morphological characters. At the outset of the work it appeared that the ratio of length to breadth might be a more useful character in the differentiation of the strains than mere length, but the breadth measurements could not be made with sufficient accuracy by an ordinary ocular micrometer to make them of value. In the experiments we encountered a very small number of aberrant cultures, characterized by slow growth, numerous deformed spores, and greatly shortened appendages, which we attributed at the time to careless use of mercuric chloride on the part of a native helper. Our supposition was confirmed by the immediate return to normality in the following generation of the strains affected. There was no basis for throwing these cultures out of consideration, since they could not be distinguished from extremes due to other factors. Consequently they increase the range of variation in generation means of a number of strains, and afford one reason for our statement, above, that more careful work would merely result in decreasing the difference which would be significant in proving the distinctness of strains.

In all, the comparisons involved thirty-five strains—twenty-two from rubber, seven from cocoanut, three from tea, two from oil palm, and one from betel-nut palm. Two strains were measured through only four successive generations, eight through five generations, sixteen through six, seven through seven, and two through eight generations. Since the time involved was the same for all strains, it is evident that some of them required twice as long for spore production as others. The numerical data for all cultures are summarized in tables 1 and 2.

In deciding how many demonstrably different lines were represented in our set of thirty-five isolations, we were of course concerned with making decisions as to how significant were the differences of the final means for the several strains. It is obvious from simple inspection of tables 1 and 2 that more than one strain was involved, for the generation means of each single strain fluctuated within a range of variation (on the average) of somewhat less than 3 μ in spore length, whereas the total range of variation of the generation means of the whole set of thirty-five cultures was 13.8 μ. We therefore felt justified in determining the standard deviation of generation means by throwing all the deviations together into one series, calculating the plus and minus deviations of the generation means each from its own strain mean. In this manner we determined an approximate value for the standard deviation of the generation means of an indefinitely large number of generations of any given strain as 1.23 μ for spore length and 2.13 μ for appendage length. All the strains were similar enough so that these standard deviations might be applied to any strain. In order to determine the significance of the differences between strain means, the usual formula for the standard error of the difference of means was applied:

$$\epsilon_{1.2}^2 = \sigma^2 \left(\frac{1}{n_1} + \frac{1}{n_2} \right)$$

TABLE 1. *Statistical Data for Successive Generations of Thirty-five Strains of Pestalozzia from Five Different Hosts*

The number of measurements in each generation was 25 for each character, except in a very few instances, when either 15 or 50 measurements were made. Measurements are in μ.

Strain	Source	Generation	Spore Length		Terminal Appendage	
			Range	Mean	Range	Mean
1	Cocoanut; leaf spot...........	1	17–24	20.3	4–21	11.0
		2	17–23	20.2	9–21	13.8
		3	16–24	20.4	15–21	9.0
		4	16–24	19.9	3–23	10.5
		5	17–23	19.6	15–17	9.2
		6	16–21	19.0	3–21	11.6
2	Cocoanut; leaf spot...........	1	17–21	19.4	4–14	9.5
		2	19–24	21.0	4–20	9.5
		3	16–26	21.3	4–21	13.1
		4	17–23	19.9	6–20	12.9
		5	17–23	20.6	3–17	12.5
3	Cocoanut; leaf spot...........	1	17–26	22.3	17–29	22.0
		2	19–26	22.2	11–27	20.0
		3	20–24	22.3	17–31	22.2
		4	19–29	22.4	10–29	17.3
		5	19–24	21.8	7–23	15.6
		6	17–24	20.9	6–24	17.6
4	Cocoanut; leaf spot...........	1	20–29	23.5	17–27	20.1
		2	19–24	21.5	19–24	20.1
		3	19–26	21.9	14–26	19.7
		4	19–26	21.8	11–29	18.4
5	Cocoanut; leaf spot...........	1	20–29	24.4	19–32	24.4
		2	17–26	21.1	14–34	23.1
		3	17–24	22.3	13–24	20.5
		4	17–24	20.2	20–29	23.0
		5	19–26	22.2	17–27	22.1
		6	19–29	22.4	13–29	19.1
		7	17–24	20.6	11–29	19.3
		8	19–29	23.2	14–26	19.0
6	Cocoanut; leaf spot...........	1	19–26	22.0	17–29	22.6
		2	20–31	23.8	14–36	21.4
		3	19–30	22.9	16–29	22.8
		4	21–26	22.9	13–26	20.1
7	Cocoanut; milk from infected nut.......................	1	20–26	22.8	14–26	18.8
		2	20–26	23.6	13–23	17.9
		3	21–27	24.2	9–21	14.6
		4	21–29	24.2	9–20	15.0
		5	21–30	25.3	14–24	18.5
		6	20–29	24.3	9–20	14.9
8	Oil palm; leaf spot...........	1	23–29	24.7	21–36	26.3
		2	21–30	24.7	20–43	27.2
		3	21–29	24.7	17–33	24.7
		4	21–27	24.4	19–34	26.2
		5	21–29	24.6	21–33	26.8

Table I (*Continued*)

Strain	Source	Generation	Spore Length		Terminal Appendage	
			Range	Mean	Range	Mean
9	Oil palm; leaf spot	1	24–36	30.3	16–29	22.3
		2	20–29	24.8	17–31	23.4
		3	23–33	27.7	20–33	26.4
		4	24–37	29.5	21–33	26.7
		5	23–34	29.7	9–31	19.6
10	Betel-nut palm; leaf spot	1	24–32	28.3	19–39	25.1
		2	19–29	24.5	21–31	25.0
		3	19–34	25.2	16–29	22.3
		4	24–36	30.0	21–36	28.8
		5	29–36	32.3	9–31	17.4
11	Tea; gray blight of leaf	1	20–26	23.0	19–33	25.9
		2	20–24	22.1	16–33	26.9
		3	20–29	23.3	16–31	25.2
		4	20–29	24.5	19–33	24.1
		5	20–27	23.9	21–34	26.6
		6	20–29	24.3	13–34	22.3
12	Tea; gray blight of leaf	1	21–27	24.4	21–37	26.9
		2	19–29	23.8	24–34	29.8
		3	24–36	28.4	24–40	31.0
		4	21–31	27.3	17–41	26.6
		5	21–33	27.2	21–37	29.4
13	Tea; gray blight of leaf	1	26–37	30.8	24–43	35.8
		2	23–34	28.5	20–42	31.7
		3	23–36	27.4	21–37	29.0
		4	24–39	28.6	21–39	30.7
		5	27–36	30.8	21–37	29.4
		6	26–36	31.3	17–36	27.9
14	Hevea; wood of sapling above wound	1	17–23	19.8	21–36	26.5
		2	16–20	18.5	16–34	24.7
		3	21–27	23.0	14–36	22.5
		4	17–26	20.7	21–37	30.0
		5	19–26	21.8	16–36	25.3
		6	16–24	21.0	17–36	26.1
		7	17–23	20.4	16–29	21.3
15	Hevea; leaves white and dry at ends and borders	1	20–27	23.2	20–32	25.2
		2	19–23	20.9	14–31	21.9
		3	17–27	21.0	21–33	25.6
		4	19–24	21.2	16–30	22.9
		5	19–23	20.8	14–31	22.3
		6	20–27	23.0	14–33	20.4
16	Hevea; healthy young leaves	1	17–24	20.4	13–32	21.4
		2	17–24	20.4	16–29	23.2
		3	19–26	22.4	19–31	24.0
		4	21–27	23.3	16–33	22.7
		5	19–29	23.0	14–31	22.8
		6	19–27	22.9	16–30	22.6
17	Hevea; leaf spot of seedling	1	17–24	22.0	19–33	25.6
		2	17–23	21.1	17–37	23.6
		3	19–24	21.2	13–34	22.1
		4	20–29	23.4	14–36	24.1
		5	19–29	22.3	17–31	23.3
		6	17–26	22.5	14–31	23.4
		7	19–27	23.3	13–27	19.0

TABLE 1 (*Continued*)

Strain	Source	Generation	Spore Length		Terminal Appendage	
			Range	Mean	Range	Mean
18	Hevea; wood of sapling above wound	1	20–26	23.4	20–32	25.6
		2	17–23	20.6	17–36	24.7
		3	20–27	22.7	14–27	20.8
		4	20–27	22.5	14–29	20.5
		5	20–26	22.5	16–31	23.2
		6	21–29	23.8	14–27	20.5
		7	19–29	22.8	13–31	21.0
19	Hevea; bark of tapping cut	1	20–27	22.3	10–26	20.2
		2	19–26	21.7	9–21	15.4
		3	19–27	22.2	16–29	21.9
		4	21–29	23.8	16–36	21.4
		5	17–29	22.8	10–26	19.4
		6	19–24	21.7	9–26	17.7
20	Hevea; wood of sapling above wound	1	19–24	21.8	19–29	23.5
		2	16–24	20.5	14–29	21.8
		3	19–24	20.9	14–27	22.1
		4	19–26	22.7	14–27	20.5
		5	21–33	25.3	16–30	22.6
		6	19–29	23.6	6–26	14.0
21	Hevea; colorless area of young leaf	1	19–27	23.0	11–29	20.5
		2	16–24	19.9	4–20	10.0
		3	20–26	22.4	20–34	25.0
		4	19–29	23.2	14–30	20.3
		5	21–29	24.3	11–30	20.5
		6	21–29	23.9	14–30	20.8
22	Hevea; untapped bark of large tree	1	21–32	25.4	14–27	20.5
		2	20–24	21.7	16–27	20.1
		3	20–26	23.0	13–26	19.0
		4	17–26	22.5	14–31	21.8
		5	23–29	24.8	14–27	21.1
		6	17–26	22.5	14–29	20.4
		7	20–27	23.5	10–27	16.7
23	Hevea; twig of tree killed by lightning	1	19–26	23.0	17–29	23.7
		2	19–24	21.9	17–31	21.3
		3	19–27	22.5	13–27	21.0
		4	21–30	24.9	13–30	18.5
		5	20–27	23.3	10–23	16.7
24	Hevea; untapped bark of large tree	1	20–26	23.4	13–26	19.6
		2	20–24	22.9	10–21	17.5
		3	17–27	22.9	14–24	20.4
		4	20–29	23.2	14–29	19.9
		5	20–29	22.8	14–29	18.2
		6	21–29	24.9	10–21	17.7
		7	20–26	23.5	11–24	18.2
25	Hevea; twig	1	20–27	23.0	17–29	21.6
		2	21–26	23.3	14–27	22.0
		3	17–26	21.8	19–30	25.0
		4	20–29	23.4	19–31	24.9
		5	21–27	24.6	14–31	22.9
26	Hevea; wood of sapling above wound	1	19–26	21.1	19–33	23.9
		2	17–23	20.1	14–30	21.6
		3	20–27	23.0	16–33	23.9
		4	21–29	24.4	16–30	23.7
		5	20–29	24.7	14–39	23.6
		6	23–29	26.6	14–31	20.1

Table 1 (*Continued*)

Strain	Source	Generation	Spore Length		Terminal Appendage	
			Range	Mean	Range	Mean
27	Hevea; falling young leaves infected with leaf-fall fungus. .	1	20–26	21.8	20–34	27.9
		2	17–29	22.9	14–36	21.0
		3	20–29	24.5	16–30	24.8
		4	21–27	24.3	16–33	21.9
		5	21–30	24.7	16–34	22.4
		6	21–29	23.7	14–29	20.6
		7	21–29	25.5	13–21	17.9
28	Hevea; bark of large branch, killed by lightning..........	1	20–26	23.0	24–32	27.0
		2	19–26	22.2	20–57	31.4
		3	21–27	24.0	19–30	23.3
		4	21–29	25.2	14–36	22.6
		5	21–29	25.6	9–29	18.3
		6	20–30	24.7	13–34	23.1
29	Hevea; wood ten inches above old wound.................	1	21–29	23.6	20–30	24.5
		2	16–27	21.7	10–27	21.7
		3	21–30	25.6	16–30	23.1
		4	17–26	22.2	20–36	26.7
		5	21–29	25.1	20–39	25.1
		6	23–30	25.9	14–39	22.4
		7	21–30	25.5	16–33	24.0
		8	21–29	24.8	19–31	24.1
30	Hevea; falling young leaves infected with leaf-fall fungus. .	1	20–29	23.9	17–33	26.1
		2	20–29	23.6	19–36	25.9
		3	20–29	24.4	16–36	24.0
		4	23–33	25.1	17–43	25.8
		5	20–30	24.7	14–31	23.0
		6	21–31	25.0	14–43	22.9
31	Hevea; old, yellow leaves.....	1	23–29	26.3	16–32	20.2
		2	21–29	25.6	14–33	21.6
		3	21–29	25.2	17–41	23.8
		4	21–31	26.0	19–30	23.3
		5	21–29	24.2	19–36	24.4
		6	19–30	24.1	17–29	22.4
32	Hevea; young leaves of cuttings with leaf-fall disease........	1	20–27	24.5	21–29	23.7
		2	21–30	26.1	16–27	21.5
		3	21–29	25.2	17–30	22.5
		4	23–29	25.5	16–33	22.5
		5	23–30	26.3	17–27	22.7
		6	23–29	26.4	16–30	23.1
33	Hevea; healthy young leaves. .	1	24–29	26.0	10–17	12.7
		2	21–29	25.9	7–17	12.2
		3	23–31	27.1	4–17	11.9
		4	21–30	25.5	8–21	13.3
		5	24–31	27.5	9–23	15.1
		6	23–30	25.6	9–19	14.3
		7	23–37	27.3	11–20	15.8

TABLE 1 (*Continued*)

Strain	Source	Generation	Spore Length		Terminal Appendage	
			Range	Mean	Range	Mean
34	Hevea; leaf spot of mature leaves....................	1	23–32	27.5	20–29	25.2
		2	21–30	25.2	14–34	23.8
		3	21–36	28.4	21–36	27.5
		4	23–30	26.6	16–31	23.7
		5	21–31	27.4	16–31	22.2
		6	23–31	28.6	13–23	17.5
35	Hevea; leaf spot of mature leaves.....................	1	26–36	29.0	16–29	22.9
		2	24–37	29.0	16–36	26.7
		3	21–29	25.4	14–31	21.1
		4	20–29	26.1	16–34	25.7
		5	24–31	28.5	20–33	27.3

TABLE 2. *Summary of Statistical Data (All Measurements and Standard Deviations in μ)*
Part 1. Spore Length

Strain	No. of Generations	Total Range of Variation	Range of Generation Means	Mean	Standard Deviation	Coefficient of Variation
1......	6	8.0	1.4	19.9	1.88	9.5
2......	5	10.0	1.9	20.6	1.85	9.0
3......	6	12.0	1.5	22.0	1.89	8.6
4......	4	10.0	2.0	22.0	1.92	8.7
5......	8	12.0	4.2	22.0	2.11	9.6
6......	4	12.0	1.8	22.9	2.33	10.2
7......	6	10.0	2.5	24.2	1.98	8.2
8......	5	9.0	0.3	24.6	2.03	8.3
9......	5	17.0	5.5	28.3	3.60	10.3
10......	5	17.0	7.8	28.0	3.90	13.9
11......	6	9.0	2.4	23.4	2.23	9.5
12......	5	17.0	4.6	26.4	3.10	11.7
13......	6	16.0	3.9	29.4	3.31	11.3
14......	7	11.0	4.5	20.7	2.16	10.4
15......	6	10.0	2.4	21.6	2.01	9.3
16......	6	12.0	2.9	22.0	2.34	10.6
17......	7	12.0	2.3	22.3	2.17	9.8
18......	7	12.0	3.2	22.4	2.00	8.9
19......	6	12.0	2.1	22.5	2.28	10.1
20......	6	17.0	4.8	22.5	2.82	12.5
21......	6	13.0	4.4	23.0	2.51	11.0
22......	7	15.0	3.7	23.0	2.17	9.4
23......	5	10.0	3.0	23.2	2.34	10.1
24......	7	12.0	2.1	23.3	2.00	8.6
25......	5	12.0	2.8	23.3	2.02	8.7
26......	6	12.0	6.5	23.5	2.94	12.5
27......	7	13.0	3.7	23.9	2.35	9.8
28......	6	11.0	3.4	24.2	2.34	9.7
29......	8	14.0	4.2	24.3	2.67	11.0
30......	6	13.0	1.5	24.5	2.28	9.3
31......	6	12.0	2.2	25.2	2.27	9.0
32......	6	10.0	1.9	25.9	2.11	8.1
33......	7	16.0	2.0	26.3	2.38	9.1
34......	6	15.0	3.4	27.3	2.56	9.4
35......	5	16.0	3.6	27.3	2.82	10.3

Part 2. Appendage length

Strain	No. of Generations	Total Range of Variation	Range of Generation Means	Mean	Standard Deviation	Coefficient of Variation
1......	6	22.0	4.8	10.9	4.54	41.6
2......	5	19.0	3.6	11.6	4.60	39.7
3......	6	26.0	6.6	18.9	4.42	23.3
4......	4	18.0	1.7	19.7	3.42	17.4
5......	8	23.0	5.4	21.3	3.96	18.1
6......	4	23.0	2.7	21.6	3.69	17.1
7......	6	17.0	4.2	16.4	3.47	21.2
8......	5	26.0	2.5	26.3	4.45	16.8
9......	5	24.0	7.1	23.7	4.56	19.2
10......	5	30.0	11.4	23.6	5.86	24.8
11......	6	21.0	4.6	25.2	4.78	19.0
12......	5	24.0	4.4	28.7	4.15	14.5
13......	6	26.0	7.9	30.0	4.73	15.8
14......	7	23.0	8.7	25.1	5.05	20.1
15......	6	19.0	5.2	23.0	4.03	17.5
16......	6	20.0	2.6	22.8	3.93	17.2
17......	7	24.0	6.6	22.9	5.33	23.3
18......	7	23.0	5.1	22.0	4.13	18.8
19......	6	27.0	6.5	19.4	4.22	21.8
20......	6	24.0	9.5	20.6	4.13	20.1
21......	6	30.0	10.8	19.4	6.38	32.9
22......	7	21.0	5.1	19.9	3.95	19.9
23......	5	21.0	7.0	19.9	4.60	23.1
24......	7	19.0	2.7	18.7	3.45	18.5
25......	5	16.0	3.4	23.5	4.09	17.0
26......	6	25.0	3.8	22.7	4.36	19.2
27......	7	23.0	10.0	21.5	4.44	20.7
28......	6	48.0	13.1	24.2	5.05	20.9
29......	8	29.0	5.0	24.0	3.96	16.5
30......	6	29.0	3.2	24.6	4.96	21.6
31......	6	27.0	2.8	22.9	4.25	18.6
32......	6	17.0	2.2	22.6	3.36	14.9
33......	7	19.0	3.9	13.4	3.10	23.1
34......	6	23.0	10.0	23.3	5.01	21.5
35......	5	22.0	6.2	24.6	4.93	20.0

The least number of generations upon which any strain mean was based was four, the greatest, eight. The least accurate comparisons, therefore, would be between two strains both of which had been carried only four generations. Conversely, the most accurate comparison would be between strains which had both been carried eight generations. The standard errors of the differences between the means of the several strains were multiplied by three, and if an observed difference between two strains exceeded this value, the difference in question was considered to demonstrate the probable distinctness of the two strains. For spore length 1.8 μ was considered a significant deviation in the case of the most dependable comparisons; 2.7 μ in the case of the least dependable. The corresponding criteria for the appendage lengths were 3.1 μ and 4.5 μ. It must be remembered that a criterion of three times the standard error of a difference between means secures practical certainty of the distinctness of strains. On the contrary, it still remains highly probable that unlike strains remain in association in the unresolved groups, which might be resolved further by more careful work.

Turning now to an analysis of the data presented in the tables, it appears that there are two strains, nos. 13 and 33, each of which cannot be like any other strain in the series. Two others, nos. 1 and 2, fall together and cannot be thrown with any other group. No. 12 can be associated only with no. 35. However, nos. 9, 10, and 34 form a natural group, with which no. 35 can also be associated. The latter, falling between no. 12 and the group composed of nos. 9, 10, and 34, can be placed with either, and it is of course a matter of judgment where it should go. No. 12 has the appendages consistently longer than the spores, whereas in nos. 9, 10, 34, and 35 the appendages are consistently shorter than the spores. A group consisting of nos. 9, 10, 34, and 35 is therefore adopted, leaving no. 12 to stand alone.

Applying our criteria to nos. 34 and 35, we find that they might have been associated with nos. 31 and 32 if they had not been placed with nos. 9 and 10. We have therefore left nos. 31 and 32 to be associated with other strains, which prove to be nos. 8, 28, 29, and 30. We might therefore segregate a group consisting of these six strains. Comparing the generation means for spore length and appendage length, however, we find that there is decided justification for considering that such a group would constitute an unnatural assemblage. In nos. 31 and 32 the spores are consistently, generation after generation, longer than the appendages; in no. 8 the exact contrary is the case; in nos. 28, 29, and 30 the spores and appendages are of the same mean length. Such consistent correlations cannot be looked upon as due to mere chance, and we are therefore forced to consider nos. 31 and 32 as constituting one group, and no. 8 another. Nos. 28, 29, and 30 come next to no. 7 in spore length, and this form proves distinct on the basis of appendage length. The next shorter strain, no. 27, proves to be distinct from no. 32 as to spore length. We have no choice, then, other than to consider nos. 28, 29, and 30 as a group.

The remaining twenty strains offer more difficult problems. Probably no two persons would group them in exactly the same way, but the results would be very similar. Tabulating the possible likenesses brings out the fact that there are three outstanding strains which are difficult to associate with any considerable number of others. These are nos. 7, 11, and 14. No. 7 falls just on the border of an otherwise relatively uniform group consisting of nos. 3, 4, 19, 21, 22, 23, and 24, and may be thrown with them or kept apart, as seems preferable. We consider it a distinct strain. Nos. 26 and 27 fall together, and cannot be combined with the large group last mentioned because of the significant difference between nos. 26 and 24. Nos. 11 and 25 are not particularly alike, but the data do not show a significant difference between them. Nos. 5, 6, 15, 16, 17, 18, and 20 form a relatively uniform group. No. 14 is best kept by itself. If it is thrown into one of the other groups it necessitates the removal of some other strain or strains from that group, and on the whole no. 14 seems the most outstanding one to be left outside the group.

This rough analysis shows us that our 35 strains fall into the following fourteen groups:

I. Strains 1 and 2, from leaf spot of cocoanut palm. Spores about 20.2 μ; appendages 11.2 μ.

II. Strains 3 and 4, from leaf spot of cocoanut palm; nos. 19, 22, and 24, from bark of trunk of Hevea; nos. 21 and 23, from leaf and twig of Hevea, respectively. Spores about 22.7 μ; appendages 19.4 μ.

III. Strains 5 and 6, from leaf spot of cocoanut palm; nos. 15, 16, and 17, from Hevea leaves; nos. 18 and 20, from wood of Hevea sapling above wound. Spores about 22.2 μ; appendages 22.0 μ.

IV. Strain 7, from milk of infected cocoanut, drawn from fruit under sterile conditions. Spores about 24.2 μ; appendages 16.4 μ.

V. Strain 8, from leaf spot of oil palm. Spores about 24.6 μ; appendages 26.3 μ.

VI. Strain 9, from leaf spot of cocoanut palm; no. 10, from leaf spot of betel-nut palm; nos. 34 and 35, from leaf spot of Hevea leaves. Spores about 27.7 μ; appendages 23.8 μ.

VII. Strains no. 11, from gray blight of tea (leaf), and no. 25, from Hevea twig. Spores about 23.3 μ; appendages 24.3 μ.

VIII. Strain 12, from gray blight of tea (leaf). Spores about 26.4 μ; appendages 28.7 μ.

IX. Strain 13, from gray blight of tea (leaf). Spores about 29.4 μ; appendages 30.0 μ.

X. Strain no. 14, from wood of Hevea sapling above wound. Spores about 20.7 μ; appendages 25.1 μ.

XI. Strains 26 and 27, from wood of sapling Hevea and young leaf of Hevea, respectively. Spores about 23.7 μ; appendages 22.1 μ.

XII. Strains 28, 29, and 30, from bark of large Hevea branch, killed by lightning, wood of Hevea sapling, and young Hevea leaf, respectively. Spores about 24.3 μ; appendages 24.3 μ.

XIII. Strains 31 and 32, from Hevea leaves. Spores about 25.5 μ; appendages 22.8 μ.

XIV. Strain 33, from young Hevea leaf. Spores about 26.2 μ; appendages 13.4 μ.

SUMMARY

1. The tropical Pestalozzias of such hosts as the cocoanut palm, Hevea, tea, etc., have not been critically differentiated by past authors, the names *P. palmarum* and *P. Guepini* being used according to individual preference for one or the other. These supposed species are incapable of definition.

2. Statistical studies of thirty-five isolations from cocoanut palm, oil palm, betel-nut palm, Hevea, and tea, show that numerous strains, morphologically distinct from one another, may be isolated, and that these strains do not appear to be confined to particular hosts.

3. It appears that by using a sufficiently refined technique a nominal species such as *Pestalozzia Guepini* might be resolved into an indefinite number of demonstrably distinct strains, the number depending only upon the precision of the methods. In illustration, we have shown a possible allocation of our thirty-five strains to fourteen groups, each of which contains one or more strains that cannot be placed in any other group.

4. The species concept in the Fungi Imperfecti is of necessity a highly artificial one. The ultimate unit, an impracticable one for purposes of classification, is the pure line descended from a single spore.

REFERENCES

1. **Stakman, E. C., and Levine, M. N.** Effect of certain ecological factors on the morphology of the urediniospores of *Puccinia graminis.* Jour. Agr. Res. **16**: 43–77. 1918.
2. **Rosenbaum, J.** Studies of the genus Phytophthora. Jour. Agr. Res. **8**: 233–276. 1917.
3. **Burkill, I. H.** The treatment to which the Para-rubber trees of the Botanic Gardens, Singapore, have been subjected. Gard. Bull. Straits Settlements **1**: 247–295. 1915.
4. **Desmazières, J. B. H. J.** Notice sur quelques plantes Cryptogames nouvellement découvertes en France, et qui vont paraître, en nature, dans la collection publiée par l'auteur. Ann. Sci. Nat. Bot. II, **13**: 181–190. 1840.
5. **Cooke, M. C.** Some Indian fungi. Grevillea **4**: 114–118. 1876.
6. ——. Cocoa-palm fungi. Grevillea **5**: 101–103. 1877.
7. ——. Blights on tea and cotton. Grevillea **1**: 90. 1872.
8. **Tanaka, T.** New Japanese fungi, notes and translations. Mycologia **9**: 167–172. 1917.

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1922

President: Henry C. Cowles
University of Chicago

Vice-President: Margaret C. Ferguson
Wellesley College

Secretary: I. F. Lewis
University of Virginia, University, Va.

Treasurer: Irving W. Bailey
Bussey Institution, Harvard University

Councilors:

N. L. Britton
New York Botanical Garden

J. C. Arthur
Purdue University

C. E. Allen
University of Wisconsin

Any one actively interested in botanical work is eligible for membership. Candidates for membership must be recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including one subscription to the American Journal of Botany. Candidates proposed for membership, and approved by the Council, may receive one copy of the Journal at $4.00 per year pending their election at the winter meeting of the Society.

The Little Things That Count Big In Owning Your Own Greenhouse

Ever gone into a hat store, knowing exactly the kind and shaped hat you want; and then have some smart Alec of a salesman try to sell you the kind HE likes?

Makes a fellow madder than a wet hen, doesn't it?

Suppose, however, that same salesman had told you something about some of his hats that meant better service to you—more satisfaction for your money—you then sort of feel towards that salesman as you would an old friend.

Salesman number two is our sort when it comes to selling a greenhouse.

We do sell greenhouses, but we first sell satisfaction.

Catalog for the asking.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

Vol. IX. MARCH, 1922 No. 3

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

CONTENTS

Undercooling of peach buds . . . . EARL S. JOHNSTON 93
Significance of the behavior of sensitive stigmas . . . . F. C. NEWCOMBE 99
The relation of age to size in certain root cells and in vein-islets of the leaves of Salix nigra Marsh . . . . MARJORIE A. TELLEFSEN 121
The origin of new varieties of Nephrolepis by orthogenetic saltation II. Regressive variation or reversion from the primary and secondary sports of bostoniensis . . . . R. C. BENEDICT 140

Smithsonian Institution
APR 25 1922
National Museum

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

Devoted to All Branches of Botanical Science

Established 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. Allen, Editor-in-Chief,
University of Wisconsin

William Crocker,
The Thompson Institute For Plant Research, Inc.

H. H. Bartlett,
University of Michigan

C. Stuart Gager, Business Manager
Brooklyn Botanic Garden

Arthur H. Graves,
Brooklyn Botanic Garden

R. A. Harper,
Columbia University

I. F. Lewis,
University of Virginia

C. L. Shear, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Postage to all foreign countries, 20 cents a volume on annual subscriptions. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume, post free.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Ffty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence. including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

Vol. IX March, 1922 No. 3

UNDERCOOLING OF PEACH BUDS

Earl S. Johnston

(Received for publication June 8, 1921)

One method of determining hardiness of fruit buds is that of estimating the percentage of injury after the buds have been subjected to low temperatures under natural or artificial conditions. West and Edlefsen[1] give a table compiled from the results of other workers showing the danger points for various kinds of fruit at three different developmental stages. The danger point for peaches at the time the petals are closed, but are just showing color, varies from 20° to 29° F. Their own very extensive studies show that Elberta peach buds in full bloom are safe at 29° F. and above. They state that occasionally temperatures of 26°, 27°, and 28° F. do no damage, but that usually one of 28° F. kills from one fourth to one half the buds, and a temperature of 22° F. kills nine tenths, while one of 18° F. fails to kill all of them. The method of estimating the injury is not very satisfactory in cases where the *degree* of hardiness is to be determined. It is very desirable that some convenient physical measurement of hardiness of fruit buds be worked out in order to ascertain more definitely the relation between the resistance offered by such buds to low temperature and various environmental conditions such as moisture, temperature, fertilizers, etc. Johnston[2] suggested that the ratio of water content to dry weight of fruit buds might serve as a possible index, but sufficient work has not yet been done to warrant its general use. Experimental results of various investigators have not always been in agreement regarding the value of the freezing-point depression of expressed saps as an indication of hardiness. Furthermore, there are a number of important conditions necessarily neglected in measuring the undercooling of expressed saps. For example, it has been shown by Bigelow and Rykenboer[3]

> That decidedly greater supercooling can be produced in capillary tubes than in tubes of larger diameter.

---

[1] West, F. L., and Edlefsen, N. E. Freezing of peach buds. Jour. Agr. Res. **20**: 655–662. 1921.

[2] Johnston, E. S. An index of hardiness in peach buds. Amer. Jour. Bot. **6**: 373–379. 1919.

[3] Bigelow, S. L., and Rykenboer, E. A. Capillary phenomena and supercooling. Jour. Phys. Chem. **21**: 474–512. 1917.

The Journal for February (**9**: 47–92) was issued March 20, 1922.

The size and shape of the capillary water films within the plant tissues undoubtedly have a marked influence on the amount of undercooling necessary to start crystallization. Also, the amount of water held by imbibition depends on the colloidal properties of the bud tissues. The degree of hardiness is so modified by these conditions that undercooling and freezing-point measurements of expressed saps do not in all probability indicate a true index of hardiness.

Numerous theories[4] have been advanced concerning the mechanical cause of injury due to low temperatures. Whether the harmful results come from failure of the protoplasm to regain water lost when ice crystals form or from precipitation of proteins or from other metabolic changes accompanying low temperatures, there is evidence that the injurious effects come at the time that ice crystals form or shortly thereafter. It must not be understood, however, that injury always results from water crystallization alone. Experiments were carried out during the early part of 1921 (January to March) with the idea of comparing the undercooling of sap within fruit buds of two peach varieties. The method used was somewhat similar to that described by Harvey.[5] A copper-constantan thermo-junction made of number 40 wires and encased in a small glass tube (0.4 mm. diam. by 3.0 mm. long) was cemented to the end of a piece of hard rubber tubing. When temperature measurements were made this junction was inserted into the upper end of a detached bud and lowered into a double-walled chamber surrounded by a freezing mixture of ice and salt. The bud was so suspended as to be free from contact with the walls of the cooling chamber. The cooling chamber was of such a size that it fitted very conveniently through the cork stopper of a pint thermos bottle containing the freezing mixture. The constant-temperature junction was placed in a similar thermos bottle filled with melting ice. This constant-temperature junction could very easily be kept at 0° C. for two days. A galvanometer similar to that described by Shreve[6] was calibrated and used to measure the temperature differences of the junctions. The copper leads were connected directly to the binding posts of the galvanometer so that a continuous reading could be made. Between each two successive readings the zero point on the galvanometer scale was obtained by a key connected directly with the binding posts. A temperature difference of 0.1° C. could very easily be detected.

In the experiments here reported fruit buds from two trees (Elberta and Greensboro) were studied. These trees, located near each other in a nine-acre peach orchard, were growing under very similar conditions.

---

[4] A brief review of a number of these theories and a bibliography of 50 titles dealing with the hardening process in plants is given by Dr. R. B. Harvey in "Hardening process in plants and developments from frost injury." Jour. Agr. Res. **15**: 83–111. 1918.

[5] Harvey, R. B. Importance of epidermal coverings. Bot. Gaz. **67**: 441–444. 1919.

[6] Shreve, Edith B. A thermo-electrical method for the determination of leaf temperature. Plant World **22**: 100–104. 1919.

Data showing the average temperature at which crystallization began and the average temperature immediately after crystal formation are designated in table 1 as "Undercooling" and "Freezing point" respectively. The temperature of the cooling mixture was not exactly the same on any two days, thus making some measurements taken on different days not strictly comparable, but the data of the two varieties on any one day are comparable. All the measurements were made in the laboratory shortly after the twigs had been cut from the trees. Twigs of similar size and similarly located on the trees were selected. The lower ends of these twigs when brought to the laboratory were cut off under water and placed in bottles of water in order to minimize the danger of drying out. Buds from the two varieties were alternately measured in pairs as a further precaution for securing comparable data between varieties. The time usually required for a bud to undercool was about two minutes, although this varied somewhat with the temperature of the freezing mixture. These same data are represented graphically in figure 1.

TABLE 1. *Data Showing the Temperature of the Freezing Mixture, the Average Temperature at which Crystallization began (Undercooling), and the Average Temperature Immediately after Crystal Formation (Freezing Point), together with the Ratio of Water Content to Dry Weight of Fruit Buds of the Elberta and Greensboro Peach.*

Date	Temperature of Freezing Mixture	Undercooling		Freezing Point		Ratio of Water Content to Dry Weight	
		Elberta	Gr'boro	Elberta	Gr'boro	Elberta	Gr'boro
1921	*Deg. F.*	*Deg. F.*	*Deg. F.*	*Deg. F.*	*Deg. F.*		
Jan. 21	14.0	17.8	18.5	21.0	20.5	....	....
Jan. 26	15.8	19.2	19.6	22.1	21.6	0.89	0.82
Feb. 12	4.3	17.8	17.6	20.3	19.6	1.03	0.94
Feb. 18	7.5	18.1	18.7	21.6	21.0	1.20	1.05
Feb. 25	8.6	18.5	18.9	21.6	21.7	1.21	1.10
Mar. 5	6.4	19.0	19.2	22.8	22.3	1.61	1.31
Mar. 11	3.2	19.4	20.1	24.4	24.8	2.53	2.01
Mar. 14	2.8	22.3	20.5	24.6	24.4	2.87	2.57

In figure 1 the lower pair of graphs represents the undercooling and the upper pair the freezing point. From February 12 to March 14 each set of graphs shows a decided rise. This indicates that the sap in these buds as they continue to develop has a higher freezing point and that it can be undercooled to a less extent. The data of the first two observations are scarcely comparable with those of later dates because of the great difference in temperature of the freezing mixtures, the rate of cooling being slower in the former cases than in the latter. There is very little difference between the freezing points of buds of these two varieties. Likewise there is but little difference in their undercoolings. Elberta, which is considered the less hardy variety, has in most cases a slightly lower undercooling point, which is just the opposite of what might be expected. On March 14, however, shortly before the petals opened, the extent of undercooling is 22.3° F.

as compared with 19.4° F. three days previous. The undercooling value of the Greensboro buds rises but 0.5° F. during the same period. On March 14 the Elberta buds were about a day further developed than the Greensboro, as indicated by the difference in time required for their petals to open when placed in the greenhouse. Whether or not these undercooling measurements mean that Elberta and Greensboro fruit buds are of practically the same degree of hardiness from January 21 to within a few days of the time the petals open can not be determined from the scant data of this one year.

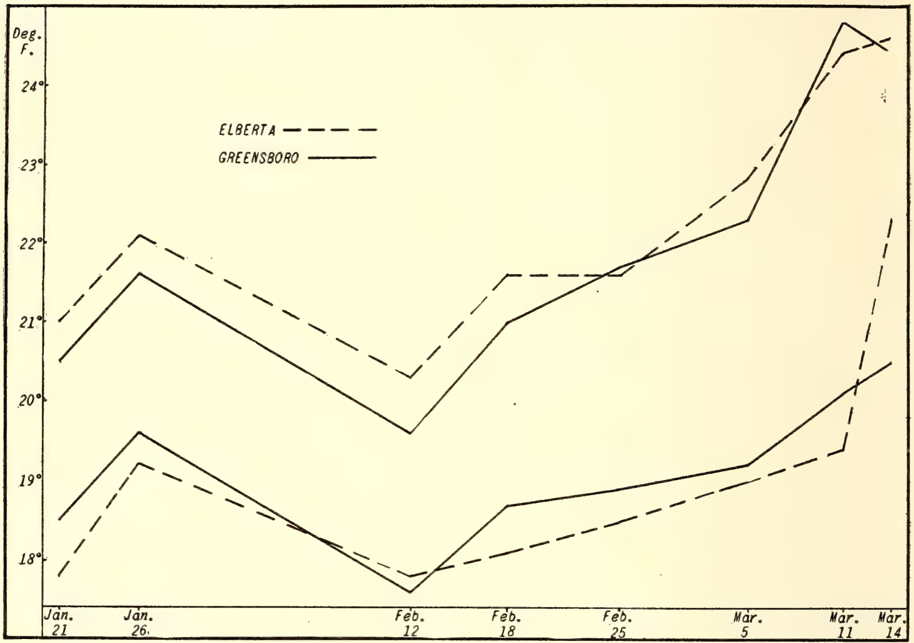


FIG. 1. Graphs showing variation in the freezing point (upper pair) and undercooling (lower pair) of fruit buds in the Elberta (broken lines) and the Greensboro (continuous lines) peach.

The extent of undercooling is probably a more important index than the actual freezing point. The freezing point in all probability is a more constant value depending largely on the concentration of the cell sap, while the extent of the undercooling is further conditioned by agitation of plant tissues due to wind movement, size of capillary films, rapidity of cooling, etc. The undercooling here obtained in the laboratory is not necessarily the undercooling that would occur outside under natural conditions. In the former case the cooling was very rapid as compared with similar changes under the usual natural conditions. Neither must these undercooling temperatures be confused with the actual killing temperatures. As a matter of fact, the actual minimum temperature[7] between February 18 and

---

[7] These temperatures were recorded by Mr. Thomas H. White, who has charge of the local U. S. Weather Bureau Station.

25 was 13° F., while the undercooling in the laboratory on February 18 was 18.1° F. for the Elberta buds. This temperature of 13° F. apparently did not injure the buds in the orchard at that stage of their development. The peach blossoms on the trees in this same orchard were, however, practically all killed on March 29 and 30 by temperatures of 25° and 20° F. respectively. These higher temperatures came at a time when the buds were in a more tender condition. If the experiment here reported could have been continued, it would undoubtedly have shown a further rise in the undercooling graphs.

Some of the buds from twigs cut on March 14 were dipped into water and then frozen while a thin film of water adhered to the bud-scales. These data and those of Greensboro buds sprayed and unsprayed are shown in table 2. Practically no difference is seen in either the undercooling or the freezing points of the sprayed and unsprayed Greensboro buds. The Greensboro tree used throughout this series of experiments had been sprayed with lime-sulphur solution on the day previous to this particular experiment. The unsprayed Greensboro was growing near by. Buds of both varieties show a greater undercooling when in the dry condition than when wet. Crystallization seemed to be more rapid and complete in the case of wet buds, as is indicated by the higher temperatures obtained in the freezing-point columns. These data obtained from dry and wet buds, although meager, indicate that wet buds freeze at a higher temperature than dry buds. This is in agreement with the results obtained by Harvey,[8] and by Chandler[9] who states that

TABLE 2. *Data Showing the Undercooling and Freezing Point of Dry and Wet Buds, and of Sprayed and Unsprayed Buds*

Condition of Buds	Undercooling		Freezing Point	
	Elberta	Greensboro	Elberta	Greensboro
	Deg. F.	*Deg. F.*	*Deg. F.*	*Deg. F.*
Dry	22.5	19.9	25.0	25.2
Wet	23.4	22.3	29.5	29.3
Sprayed	....	20.1	....	24.8
Not sprayed	....	20.3	....	25.0

> Tissue with a wet surface killed worse at a given temperature than did tissue with no moisture on the surface.

West and Edlefsen[10] in their orchard experiments of 1912 likewise found that wet buds and blossoms were killed at low temperatures which did no injury to similar buds and blossoms in a dry condition.

---

[8] Harvey, R. B. *Op. cit.*

[9] Chandler, W. H. The killing of plant tissue by low temperature. Univ. Mo. Agr. Exp. Sta. Res. Bull. 8. 1913.

[10] West, F. L., and Edlefsen, N. E. Orchard heating. Utah Agr. Coll. Exp. Sta. Bull. 161. 1917.

SUMMARY

By means of a thermo-electrical method the extent of undercooling of Elberta and Greensboro fruit buds exposed to the low temperatures of freezing mixtures was determined.

The data obtained indicate an increase in the tenderness of these buds with the approach of spring, and that just before the petals opened the buds of the Greensboro variety withstood a greater undercooling than those of the Elberta.

Other data obtained indicate that wet buds freeze at a higher temperature than dry buds. A period of cold weather immediately following a rain is thus apparently more dangerous to fruit buds of the peach than cold weather alone.

LABORATORY OF PLANT PHYSIOLOGY,
UNIVERSITY OF MARYLAND AGRICULTURAL EXPERIMENT STATION

SIGNIFICANCE OF THE BEHAVIOR OF SENSITIVE STIGMAS [1]

F. C. Newcombe

(Received for publication June 11, 1921)

It has been known for a long time that in the families of the Bignoniaceae, Scrophulariaceae, Martyniaceae, and Lentibulariaceae there are certain genera whose stigmas respond to pressure. In nature, the stimulating object is the body of a visiting insect or of a humming bird. The external structure of these sensitive stigmas is similar, though not closely similar, in all species. The stigma consists, in most of the species, of two tongue-shaped lobes which ordinarily diverge 180° or more. The pollen is deposited on the inner surface of these gaping lobes by the visiting insect, and the push of the insect's body stimulates the lobes to close together completely, except in the Lentibulariaceae, in which there is but partial closing.

I. The Primary Closing of Stigmas

1. Stimuli Causing Primary Closing

As stated above, the primary closing of stigmas includes that striking behavior in which the two divergent stigma lobes close together as a result of the pressure of an insect or other body, the response being therefore thigmotropic.

As long ago as 1841, sensitive stigmas were described by Henderson (1), and in 1861 by Kabsch (2). More recently some dozen authors have published their observations and experiments, till, at the present time, we know some two dozen species and varieties with sensitive stigmas. A list of these species, with the names of authors publishing them, will be found under the "Summary," at the end of this paper.

The notion that the stigmas are anywhere sensitive (or responsive) to the slight pressure called *touch* is erroneous, as shown by Lloyd (14) for *Diplacus glutinosus*, and as I have found for six species I have tested. A human hair may be glued to a style, leaving two centimeters free, and with the tip of the hair one may stroke the stigmas anywhere without causing closing. I have commonly used this instrument in the pollination tests, the hair carrying the pollen very well.

To locate the sensitive region of the stigma, various authors have explored the stigma lobes with various instruments. Heckel (8) found both stigma lobes equally sensitive, but the inner surfaces of the lobes more sensitive than the outer surfaces. Oliver (10), working in part with the same species as Heckel, found the stigma lobes sensitive on the inner faces only. Burck (12) found both surfaces sensitive, but the inner surface the more so. Lloyd (14), by probing the stigma of *Diplacus glutinosus* with a dull-pointed glass rod, was convinced that the lobes are sensitive on their inner faces only, and that a response is brought about only when the cells of the inner faces are stretched by the bending of the lobes.

---

[1] Contribution 184 from the Botanical Department of the University of Michigan.

My own work in determining the location of the sensitive area of the stigma has been done on *Catalpa bignonioides*, *Tecoma radicans*, *Torenia fournieri*, and *Utricularia vulgaris*. In Catalpa, the lobes are sensitive on both the inner and outer surfaces, though more sensitive on the inner. In the other three species, the lobes are sensitive on their inner surfaces only. To determine this result, the following method was used, except for Utricularia, which has but one sensitive lobe, the other being small and not motile. The flower was secured with its pedicel between the halves of a cork held by a clamp. The corolla and stamens were then removed. Next, one lobe of the stigma was seized by a fine forceps and bent back out of the way of the other lobe, the object being to prevent the two lobes coming into contact during the manipulation. With any kind of a blunt probe, one may now explore the free lobe for the sensitive region. By this method, the lobes of Tecoma, Torenia, and Utricularia may be pushed about at will—from 20 to 50 flowers were used for each species—as long as the probe is against the outer surface only; or percussion may be used on the outer surface without bringing a response. The same sort of treatment will, however, cause the prompt closing of the stigmatic lobes of Catalpa. The sensitiveness of Catalpa to pressure on the outer faces of the stigmatic lobes shows that the condition for reaction that Lloyd found for *Diplacus glutinosus*—the stretching of the cells of the inner face of the stigma—does not obtain for all species; for one can hardly believe that pressure on the back of the lobe will cause the elongation of cells on the inner face. The question must be left open, however, as to whether the stimulus on both the outer and the inner faces of the stigma of Catalpa is due to pressure or to stretching of cells on the respective faces.

That the body giving the stimulus need not be solid has been shown by obtaining the same responses when using a fine jet of air against the stigma lobes of *Tecoma radicans*, *Torenia fournieri*, and *Utricularia vulgaris*.

Besides the pressure stimulus, there is no doubt that an electric current through the stigmas may induce the primary closing. Kabsch (2) notes this for *Mimulus guttatus*, and I have had the same result with *Catalpa bignonioides*. Heckel (8) records the vapors of hydrocyanic acid, acetic acid, ammonia, chloroform, ether, etc., as inducing primary closing, and Correns (11) found the same for ammonia vapor and reduced oxygen pressure. Lloyd (14), however, showed that heat, alcohol vapor, and liquid alcohol cause the closing of the stigma of *Diplacus glutinosus* only as they kill the stigma. I have found the same relations in the behavior of the stigma of *Catalpa bignonioides* toward ammonia vapor. By normal pressure stimulus, my Catalpa in first class condition at 29° C. will begin closing in 2 seconds and completes its closing in 7 seconds from the moment of stimulation. In strong ammonia vapor, at 29° C., the stigmas began closing in 15 to 20 minutes. One flower, on removal from the vapor after a 5 minutes' stay, showed its stigma normally sensitive to pressure. The stigmas of the other flowers left in the ammonia vapor were still closing slowly 55 minutes after being placed in the vapor, and were completely closed and dead 70 minutes after being placed in the vapor. Lloyd's results with heat and alcohol and my own with ammonia vapor raise the question whether Heckel's (8) results, obtaining closing with these same agents and others, were really sensitive responses, as he thought, or rather the result of the death of the protoplasm. Of course, one must bear in mind the fact that many contractile organs, like the leaves of Mimosa, respond in a sensitive way to ammonia vapor and to other poisonous vapors and gases; so that, *a priori*, one might expect the stigmas to close in the presence of various vapors and gases.

Another method of stimulating for *Catalpa bignonioides* consists in crushing a portion of the style. Burck (12) states that the styles of the species he worked with could be cut through without causing the stigma to close. Many trials of the same kind were made with the Catalpa I worked with and no closing ensued, if the knife was sharp, no matter where the style was cut. If, however, scissors were used, and the cut was not more than 2.5 millimeters from the insertion of the stigma lobes, the most sensitive stigmas at 30° would close after the lapse of 5 to 15 seconds. Crushing the style with forceps whose tips were one millimeter wide was a much surer way of causing closing. In one series of 21 pistils, the style crushed with the forceps 10 mm. from the insertion of the lobes, temperature 29°, the stigmas closed at periods 15 to 120 seconds from the time of crushing. In another series of 10 pistils, style crushed as above noted at a distance of 2.5 mm. from the insertion of the lobes, temperature 29°, closing took place in 5 to 16 seconds from the time of crushing. These two series with their results are fairly representative of the whole number of tests. Pinching the style between finger and thumb will often, but not always, cause the stigmas to close. As a result of the pinching, no injury to the style could be detected—no deformation nor infiltration.

When one considers the time of the response when the style was crushed at 10 millimeters, and again at 2.5 millimeters from the stigma lobes, it would seem that the closing, probably due to sudden increase of water-permeability in the cells of the stigma lobes, must have been induced by an impulse received from the injured protoplasm of the style. The change in permeability of the contracting cells in the stigma could hardly be due to the sudden flow of water toward the crushed spot of the style, for there was no closing when the style was cut through with a razor; nor could it be due to the forcing of cell sap into the stigma when crushing the style, for the response came too long after the crushing to be so accounted for.

The stigma of *Mimulus glabratus* var. *Jamesii* also could be stimulated by crushing the style 2 millimeters from the insertion of the lobes, though the closing was never complete, and the lobes started to retrace their course 2 to 3 minutes after the crushing of the style.

2. Relative Sensitiveness and Transmission of Stimuli in Various Species

Heckel (8) attempted to arrange the 9 species that he studied in a series according to the sensitiveness of the stigma, citing Martynia and Mimulus as among the most sensitive and Tecoma, Bignonia, and Catalpa among the least sensitive. The Martynia stigmas he saw complete their closing in 3 seconds after stimulation, while the stigmas of the 3 genera named above as less sensitive required 60 seconds.

During the several years of my observations, I have watched the closing of thousands of stigmas, with not fewer than scores of observations in any of four genera and nearly a score in each of two species in another genus (Mimulus). Though I have made no special study of the relative reaction time of various species, I am certain that the response of *Catalpa bignonioides* is, under favorable conditions, completed in 7 seconds, that of *Torenia fournieri* in 2 seconds; of *Tecoma radicans* in 3 seconds at 30°; of *Mimulus glabratus* var. *Jamesii* in 10 seconds at 21°; while even the slowest of the eight species I have observed has shown individual closures within 12 seconds of stimulation. I have seen, on one tree of *Catalpa bignonioides* growing in good soil, stigmas close in 7 seconds, while on another tree, stunted and in poor dry soil, the stigmas required 60 seconds or more to close. The following season, flowers were found on the latter tree whose stigmas closed in 10 seconds. The responsiveness of the stigmas, as is the case with sensitive reactions generally, is so influenced by external and internal conditions, that only a special study can determine the relative sensitiveness of the stigmas of species; and such a study has not yet been made.

Perhaps it is remarkable that so few species of the Scrophulariaceae have been reported to have sensitive stigmas, seeing that there are so many that have the two-lobed form of stigma characteristic of those that are sensitive. It would seem probable that search will discover other species sensitive, and different degrees of sensitiveness. A little attention on my part has been given to the testing of other species for sensitiveness, but, of the several examined, only *Digitalis purpurea* and a hybrid, *Digitalis purpurea* ♀ × *D. lanata* ♂, have given any response. The response is relatively feeble in both cases. In *D. purpurea*, generally no movement of stigma lobes could be detected. On one occasion, when the stigmas of seven flowers were given the pressure always bringing a response in Mimulus and the other plants already mentioned, four stigmas showed a slight closing movement, the lobes traveling 10° to 15°. The hybrid, in 20 or more stigmas tested, showed a larger number of responses, about half the stigmas moving 20° to 80°. The stigmas of these plants never close, and the small movements noted can be of no benefit to the plant.

Quite another topic on which considerable work has been done is the transmission of stimuli. Heckel (8) simply states that the stimulus is transmitted from one lobe to another, without giving his method for determining such transmission. Burck (12) believed that the stimulus was not transmitted in the eight species he worked with. Oliver (10), by holding one lobe of the stigma with forceps so that it could not press against the other lobe, determined that the stimulus is not transmitted from one lobe to the other in *Mimulus luteus*, but is transmitted in *Mimulus cardinalis*, *Martynia lutea*, and *Martynia proboscidea*. Lloyd (14) found the stimulus not only transmitted from one lobe to the other in *Diplacus glutinosus*, but not even transmitted from one part to another of the same lobe. The stigma of *Catalpa bignonioides* also shows no transmission of stimulus from one lobe to the other, as I have proved many times by using Oliver's method; but in the lobe stimulated, the impulse travels apparently over the whole lobe from the minute area where the stimulus may be applied. In *Mimulus punctatus*, not many flowers were available for trial, but the six stigmas used showed no transmission of stimulus from one lobe to the other. In *Torenia fournieri* there is also no transmission of stimulus from one lobe to the other, as I ascertained by numerous tests.

We may thus feel fairly certain that in some species the stimulus is transmitted from lobe to lobe, while in others it is not; and that in the same genus, as in Mimulus, one species may show transmission and another may not.

3. Significance of Primary Closing

It is a matter of common observation that sensitive stigmas of all species reopen a few minutes after closing, provided that no pollen is deposited at the time of stimulation. The behavior of pollinated and closed stigmas has, however, been described by very few authors. Burck (12) states that all of the eight species he worked with, except *Torenia fournieri*, kept their stigmas continuously closed after first closing at the time of pollination; and Torenia stigmas remained closed if the pollen used on the stigmas was dry. Elrod (13) found the pollinated and closed stigmas of *Tecoma radicans* nearly always remaining closed, and always remaining closed if fertilization ensued. On the other hand, Lloyd (14) records the stigmas of *Diplacus glutinosus* as opening regularly, shortly after forcible pollination, to close again permanently some hours later.

In all of the seven species whose stigmas I have tested for opening after forcible pollination, I have found all the stigmas opening in two species and some of the stigmas opening in each of the five other species. In *Utricularia* *vulgaris* all seven flowers used opened their stigmas 20 to 65 minutes after forcible pollination. In *Mimulus glabratus* var. *Jamesii* the eight stigmas which were pollinated and closed subsequently opened, four of them within 30 minutes, and the other four probably in about the same time, though observation was not made till 10 hours after the first closing, when the four were all wide open. Having but a few flowers of *Mimulus punctatus*, I used but one stigma for the present purpose. This stigma opened 10 minutes after forcible pollination. In the species *Mimulus cardinalis* nine forcibly pollinated stigmas were observed for subsequent behavior. Eight of these stigmas opened in periods ranging from 20 to 150 minutes after closing, the earlier opening taking place in a temperature of 28.5°, the later in 12.5°. The plant used was in the open garden and was protected from insects by a cheesecloth net. In the species *Torenia fournieri*, twenty stigmas were forcibly pollinated and closed. Eleven opened in 20 to 70 minutes, and nine remained closed. This is the same species used by Burck (12) and found by him to keep its stigma closed with dry pollen and to open the stigma after moist pollen was used. In my hands the stigmas of this species opened after using either dry or moist pollen; but I shall return later in this paper to the matter of the influence of moisture on closing. Twenty-seven flowers of *Tecoma radicans* were observed for the behavior of forcibly pollinated stigmas. Twenty-six of these stigmas remained closed and only one opened. In the species *Catalpa bignonioides*, 89 stigmas were observed after being pollinated and closed by the pressure stimulation. Some of these tests were made in sunshine and some in shade, some in dry air and some in moist, and in temperatures from 18° to 32°. The general summarized result gives 39 stigmas opening after the first closing and 50 remaining continuously closed. In very moist air, the most of the pollinated and closed stigmas of Catalpa and Torenia will reopen.

Inasmuch as none of my experiments here recorded were given conditions that might not obtain in nature, it follows that the stigmas of all my seven species tested may reopen in appropriate natural conditions, after their closing at the time of pollination by the usual natural agent. One may believe that not only these seven species and the Diplacus, as found by Lloyd (14), but also all the other related species known to have sensitive stigmas may at times show their stigmas opening after the first closing, and subsequently closing again. These relations are justification for speaking, as in this paper, of *primary* and *secondary* closing of the stigmas.

As the primary closing of stigmas, even when pollinated, is in *Utricularia vulgaris* and *Mimulus glabratus* generally, and may be in other species not infrequently, followed in 15 to 60 minutes by opening, it is necessary to assume that the biological significance of the primary closing is likely to be some immediate good in the life of the plant. F. Müller (4), Hildebrand (5), Batalin (6), H. Müller (7), Darwin (9), and Elrod (13) have assumed that the closing of the stigma serves the plant in preventing its own pollen from reaching the stigma, the visiting insect causing the stigma to close before the insect reaches the pollen of the same flower. This view would seem to express the obvious significance of the phenomenon. The only objection to this interpretation has been raised by Burck (12) who states that in *Torenia fournieri* the visiting bee encounters the pollen before touching the stigma. I have examined the position of anthers and stigma in hundreds of flowers of *Torenia fournieri*, and have never seen a case in which a visiting bee would touch the pollen before the stigma. This does not imply that Burck may not have seen such cases; for the species of Torenia, Catalpa, Tecoma, and Mimulus that I have examined show considerable variation in the position of anthers and stigma, though in the great majority of cases the well known relation is present of one pair of anthers far above, the other pair below, and the stigma between the two pairs and projecting in front of them; or the stigma rises above all the anthers, and projects forward into the open throat of the flower. In flowers of *Catalpa bignonioides* and *Mimulus cardinalis*, I have seen in a few instances stigmas covered with pollen from anthers pressing in between the open stigmatic lobes. But these unusual relations cannot be cited as evidence against an hypothesis supported by the evidence of the usual behavior. F. Müller (4) found the flowers of *Tecoma* sp., Elrod (13) found those of *Tecoma radicans*, and Batalin (6) those of *Mimulus guttatus* infertile to their own pollen. On the other hand, I found *Torenia fournieri*, *Mimulus cardinalis*, and *Utricularia vulgaris* readily self-fertile, and one case of self-fertilization in *Catalpa bignonioides*.

II. The Secondary Closing of Stigmas

As stated earlier in this paper, the opening of stigmas, which often occurs after the primary closing at the time of insect pollination, has not been generally observed. The usual statement is that the stigmas remain permanently closed provided pollen has been deposited at the time of closure. This statement is true for some species only; for other species, under certain conditions there may be an opening of stigmas following the first closing, and later another closing without a second stimulation.

1. Phenomena as Observed in Nature

The species *Utricularia vulgaris* differs in its stigma structure from all other species so far reported to have sensitive stigmas, in that it has but one motile lobe. Only the lower lobe is long and motile, while the upper lobe is short and rigid. A description of this stigma may be found in Hildebrand's (5) paper. When the lower lobe of the Utricularia stigma is mechanically stimulated and pollen is deposited upon it, it immediately rises through an arc of 180° or more, but does not close against the upper lobe, there being left a small open chamber between the two lobes. This motile lobe, however, soon begins to retrace its course, so that in 20 to 30 minutes it is near or in its first position. *Mimulus glabratus* var. *Jamesii* is another species—this one with the usual two subequal and motile lobes—that, in its usual behavior, opens its stigma lobes a few minutes after the first closing at the time of pollination. Lloyd (14) found the same behavior with *Diplacus glutinosus.* We thus know of three species that, in their usual behavior, open their stigmas soon after the first closing at the time of pollination. Possibly other species will be found to behave in the same way. In preceding pages, I have reported four species whose stigmas do not always open after the first closing with pollination, but may open and later show secondary closing.

Of the 24 species and varieties now known to possess sensitive stigmas, 15 have been tested to determine whether pollen could be placed on the stigma without causing the stigma to close immediately, and with all 15 the test has been successful. In 13 of the 15 species, however, the majority of the stigmas of each species in usual weather close 2 to 5 hours after the application of the pollen. Stigmas, therefore, may show the secondary, without showing the primary, closing.

2. Significance of Secondary Closing

An extensive series of experiments in my own work with *Catalpa bignonioides* and with *Torenia fournieri,* as well as less extensive tests with several other species, have demonstrated the fact that the pollen does not germinate on the stigma, unless the stigma lobes are closed, except in unusually moist air.

A number of tests of the time of germination of the pollen of Catalpa resulted in showing that in 10 percent sugar solution at 29°, initial pollen tubes could be found after 3 hours, at 23° after 3¾ hours, good germination with long tubes after 6 hours at 23°, and 80 percent germination at 26° after 8½ hours. Pollen from freshly opened anthers germinates well, and pollen adhering to open anthers retains its viability for three days or more after dehiscence.

The great majority of stigmas of Catalpa left on the tree close whether pollinated or not. If the blossoms are brought into the house and placed in a damp chamber, some stigmas will close, but some will remain open continuously even though pollen may be placed on the lobes. In a series of eight experiments, the stigmas of 48 blossoms were cross-pollinated so as not to cause closing of the lobes, and the blossoms were kept with their stalks in water in a moderately moist chamber at 22° to 26°. Of the 48 stigmas used, 22 remained open continuously for 24 to 26 hours, when they were examined for germination of pollen. There was no germination of pollen. Eight flowers had their stigmas cross-pollinated without closing the stigmas, and the flowers, dipping into water, were kept in a chamber nearly saturated with moisture for 26 hours, at 23°. In this period none of the stigmas closed. Pollen germination as high as 75 percent was shown on all stigmas. Of the 48 stigmas mentioned above as cross-pollinated and kept in a moderately moist chamber, 26 closed or partially closed 3 to 10 hours after pollination, but not one of them remained closed continuously for the 24 hours or more of the experiment. All of these stigmas showed pollen germination estimated in the different individuals at from 10 percent to 75 percent, the percentage of germination varying with the degree of closure or with the duration of the closure of the stigmas.

The stigmas of *Torenia fournieri* proved themselves less influenced by dryness of air than did those of Catalpa. When a potted plant bearing blossoms was kept in moderately moist air, pollinated stigmas generally remained open if the pollination was so done as to avoid primary closing, or the stigmas opened in 30 to 60 minutes if they had been stimulated to close at the time of pollination. In three series of tests in which some of the stigmas had closed and so remained, while others had opened or remained open, microscopic examination at the end of 24 hours showed the pollen ungerminated on the open stigmas, but germinated on the closed ones. A more extensive series of tests was made with this same species in which the growth of ovules was used as the criterion for the growth of pollen on the stigma. In one series, during damp weather, 13 stigmas were given pollen without causing closing, the plant being kept outdoors under a net screen in natural atmosphere. The stigmas did not close, and there was no fertilization. In another test, six flowers had pollen placed on their stigmas without closing. The pot was kept on wet ground outdoors under a net-covered wire cage covered with a wet blanket nights, and with water falling on the net during the day. The plant must have been in a nearly moisture-saturated atmosphere continuously, though no water fell on the plant. The preparation was kept going for six days without closing of stigmas. At the end of this period, all six ovaries showed abundant, large ovules.

A similar test to the foregoing, except that the chamber holding the plant was kept only moderately moist, was made with six pollinated flowers. Of these, four stigmas closed 4½ to 14 hours after pollination, remained closed 12 to 14 hours, opened or partly opened for the next 3 to 10 hours, then closed permanently. The ovaries of these four showed good growth with a good supply of ovules eight days after pollination. The other two stigmas half closed about 12 hours after pollination and so remained between 7 and 10 hours, then opened and so remained. The ovaries of these two flowers after 8 days had grown but little and contained but a few enlarged ovules. The pollen used on the foregoing six stigmas was all taken from the longer pair of stamens of one flower. The flowers were constantly protected from insects by a cheesecloth net.

Nine flowers of *Tecoma radicans* were brought into the laboratory where the air was cooler and more moist than outdoors. The open stigmas were pollinated all from a mixture of pollen from two anthers of another flower. Five stigmas were caused to close at once by stimulating, and four were pollinated without closing. All the stigmas maintained, till microscopical examination the next day, their original closed or open position. The five closed stigmas showed their pollen well germinated, but the four open stigmas showed no germination of pollen.

With *Mimulus cardinalis*, only one experiment is pertinent for entry here. Four stigmas were given pollen and closed by the pressure stimulus. The plant bearing the flowers was in the garden, and was covered with a cheesecloth net. The weather for the first three days was moist and ranged from 12.5° to 21°. The first stigma remained closed throughout; 12 days after pollination the ovary was large and had abundant ovules. The second stigma was closed on pollination but opened after two and a half hours, remained open a day and then closed finally; twelve days after pollination the ovary was about two thirds full size and had about half the normal number of ovules. The third stigma was closed on pollination and remained closed throughout; examination twelve days after pollination showed a normal-sized ovary filled with ovules. The fourth stigma was closed at the time of pollination, but opened in an hour and did not close again; at the end of the twelve days, the ovary had grown to less than half the size of the preceding one, and had one fourth as many ovules. In the last case there must have been germination of some of the pollen on the open stigma. This germination could be accounted for by the moist atmosphere which prevailed for the two days following pollination.

From the results obtained with the foregoing four species, representing four genera, there can be no doubt that the continued closure or the secondary closure of the pollinated stigmas serves the plant in securing germination of pollen on the stigma. Of the eight species which have been under my examination, only *Utricularia vulgaris* has shown fluid on the stigma. Lloyd (14) reports Diplacus as having no stigmatic fluid. The value of the closure of the stigmas in promoting pollen germination would seem obvious, and the experimental results confirm the assumption.

3. Cause of Secondary Closing

As stated before in this paper, the stigmas of some of the two dozen species of plants known to possess sensitive stigmas reopen in less than an hour after they have been closed at the time of pollination. These stigmas, except in *Mimulus glabratus* and *Utricularia vulgaris*, close again within 2 to 14 hours, unless the weather is unusually damp. Some stigmas in several of the species do not open after the primary closing at the time of pollination, and hence the effect is the same as though there were opening followed by secondary closing. The question now to be considered is the cause of the continuance of the primary closure, and of the secondary closure when there has been an opening after the primary closure.

a. Effect of the Germination of Pollen on the Stigma

It cannot be the germination of pollen followed by the penetration of pollen tubes into the tissue of the stigma that causes the stigma to remain closed after the primary closing. For I have found none of the pollens, under the most favorable conditions, on the stigmas or in sugar solutions, germinating in less than two hours. Indeed, in only three tests have I found it germinating in less than six hours. The stigmas, however, of all species so far reported by others, as well as those under my own observation, open in most cases, in temperatures of 18° or above, within 30 minutes of the primary closing, provided no pollen has been placed on the stigmas. In such time relations, it may be assumed that the pollen has some effect in keeping the stigmas closed; but it cannot be the germination of pollen. Nor can it be the germination of pollen which causes the secondary closing of the stigmas; for, in the first place, many examinations of mine have shown that the pollen does not germinate on stigmas in ordinary weather till the stigmas have closed; and, in the second place, when pollen does germinate on stigmas in very moist air, the stigmas do not close.

b. Withdrawal of Water from the Stigmatic Cells

Burck (12) found that if he pollinated the stigma of *Torenia fournieri* with dry pollen and closed the stigma, the stigma remained closed; but if he used moist pollen the stigma soon opened again. From this behavior, he inferred that it was the withdrawal of water from the stigma that kept the stigma closed.

Lloyd (14), by observing the local curvature of the stigma lobes of *Diplacus glutinosus* when pollen masses were placed on different spots on the inner surfaces, came to the same conclusion as Burck.

Brown (15), using *Martynia proboscidea*, could load the stigma lobes with sand or could give them a little quartz flour without causing closing. When, however, he used a large quantity of quartz flour, the stigma closed. From this behavior, he inferred, with Burck and Lloyd, that it is the withdrawal of water from the cells on the inner side of the lobes that causes the secondary closing.

My own rather extensive work on the cause and conditions of secondary closing may be narrated under three sub-headings.

1. *Relation of closing to atmospheric moisture.* Among the several species reported in this paper, two distinguish themselves from the others in that the bilabiate corolla has a closed throat, and the plants themselves grow in water or only where the substratum is very wet. These species are *Utricularia vulgaris* and *Mimulus glabratus* var. *Jamesii.* Seven individual flowers of the former and eight of the latter were cross-pollinated and the stigmas closed, but all stigmas opened soon afterward and did not subsequently close. Nevertheless the ovules of Utricularia were fertilized; but

the behavior in Mimulus was not followed long enough to determine this matter. Yet it cannot be doubted that the pollen germinates on the open stigmas of the Mimulus and that fertilization results. Pollen germination on the open stigma of Utricularia was determined by teasing out several stigmas some hours after pollination. Besides the seven cases, specially tested as noted above, many other stigmas of Utricularia were seen to have the lower lip recurved (open) after the fall of the corolla. Yet all these flowers bore seeds.

The other species subjected to experiment and observation have open flowers and hence do not keep moisture about their stigmas sufficient, in usual weather, to promote germination. It would, perhaps, be correct to say that they do not keep sufficient moisture about their stigmas to allow, or cause, reopening after the primary closing, or to prevent the secondary closing in case there has been a secondary opening. Especially careful observations for determining these relations have been made with *Torenia fournieri* and *Catalpa bignonioides*. The general result has been the failure of secondary closing in very moist air and the occurrence of secondary closing in dry air. Six stigmas of Torenia, pollinated and not closed, kept outdoors under a fine net, weather 23° to 26°, not very moist, all closed in 4½ to 14 hours. Six other stigmas, pollinated and not closed, the plant kept day and night outdoors in a nearly moisture-saturated chamber, in good light by day, temperature 23° to 26°, did not close at all. Seven other stigmas were cross-pollinated and closed at 11 A.M. on a sunny summer's day, and the potted plants were set outdoors in the sun under a fine net. Four of the stigmas soon opened at the tips of the lobes, but closed again during the next few hours. At 7:50 P.M. six of the seven were well closed, and the seventh was half closed. The plants were then brought into the house and kept in moister air over night. In the morning, four stigmas were open, the other three closed. The stigmas of thirteen flowers had small masses of pollen placed on the stigmatic surface near the tips of the lips, and the plants were kept outdoors under a fine net. The weather was moderate and clear, fairly dry, with day temperature 22° to 28°. The stigmas were not closed at first and did not close subsequently. They were watched for two days after pollination. Besides these series of experiments, many pollinations and closings of stigmas were made, generally resulting, unless a damp chamber was employed, in the pollinated stigmas remaining closed permanently.

Generally, if the air is moist and the sky clouded, one will find the stigmas of *Catalpa bignonioides* open while the flowers are still on the tree. On the other hand, in sunshine, especially in the afternoon, the most of the stigmas are closed. The closing could be referred to the effect of the dry air, or to insect pollination, or to a combination of the two things. To determine whether the stigmas would close in usual weather, the open flowers were removed and discarded from several panicles on a part of the tree but little reached by the sun, and paper bags were tied over the panicles. The following day the bags were removed. The open flowers were then always found with open stigmas. Several panicles were brought into the laboratory and their stems were set in water. The open flowers were discarded, and a wait was made for the immature blossoms to open. When the blossoms were open and stigmas were open, the inflorescences were subjected to conditions which brought wilting, or to conditions preventing wilting. The result reached was that the stigmas close, without stimulation or the presence of pollen, as soon as the corollas show any wilting. The corollas show flagging very quickly in experimentation; and quite generally in natural conditions on the tree—such temperature and moisture conditions as obtain the latter part of June and through July, when the trees are in bloom—the corollas are found flagging and the stigmas closed. My observations on the closing of the unpollinated stigmas because of wilting and the readiness with which incipient wilting takes place in the usual behavior of these flowers have extended to hundreds of flowers and have covered three flowering seasons.

Assuming that the stigmas of Catalpa close without pollination, with incipient wilting of the flower, as shown in the preceding paragraph, one may next inquire whether the presence of pollen on the stigma will cause closing when there is no wilting. My notes show that 109 flowers have been used in trying to answer this question. It was found that if, after pollination without immediate closing of the stigma, precautions were taken to insure a very moist atmosphere, there was no subsequent closing. In an atmosphere not very moist yet moist enough to prevent wilting of the flower, the presence of pollen induced closing when in its absence there would have been no closing.

With the flowers of *Tecoma radicans*, 17 stigmas were cross-pollinated without closing the stigmas immediately. The flowers were kept in the house, their pedicels dipping into water in beakers. The conditions were, therefore, fairly moist and there was no wilting. Only one of the 17 stigmas subsequently closed. Several unpollinated flowers lay on the table without water for 10 hours, and thus were allowed to wilt; but they showed no closing of the stigma. This series of tests is incomplete. It needs still the case of pollination, without primary closing, in dry air, or with flowers on the vine, to see whether the secondary closing would ensue. That the presence of pollen on the stigma is effective in keeping the stigma closed after it has been stimulated mechanically to close was shown by Elrod (13); and numerous tests of my own confirm his results.

2. *Use of absorbent substances on the stigma.* As stated before in this paper, Burck (12), Lloyd (14), and Brown (15) have expressed the view that the secondary closing of stigmas is due to the withdrawal of water from the inner surface of the stigma lobes by the pollen. There is no question that the presence of pollen in dry air either keeps the stigma closed

after the primary closing, or induces a secondary closing if there has been a secondary opening or if there has been no primary closing. It should be recalled here that the two species *Utricularia vulgaris* and *Mimulus glabratus* var. *Jamesii* are exceptional in that the stigmas show no secondary closing. It may also be recalled that the other species reported in this paper show much oftener than hitherto supposed both secondary opening and a failure of secondary closing, especially in moist air.

Moist and dry pollen. Only 19 stigmas were used—7 of *Torenia fournieri* and 12 of Catalpa—in comparing the effect of moist and of dry pollen on maintaining the closed condition of stigmas. The result cannot be said to be conclusive, but only indicative. When the atmosphere was dry, the stigmas receiving dry pollen remained closed longer than those receiving moist pollen, or a larger proportion of the former remained permanently closed. When the atmosphere was moist, the most of the stigmas opened without distinction between dry and moist pollen. The dry and moist pollens were always used at the same time on similar flowers, the dry being taken in some cases from anthers open 24 or more hours, while the moist was taken from unopened anthers. Or, in other cases, both pollens were taken from the same anthers, open 24 or more hours, the pollen applied dry to some anthers and moistened with spring water before being applied to others.

Wheat flour. Four open stigmas of Torenia were given a fair amount of wheat flour, and closed. The plant bearing the flowers was kept in the house where the moisture was considerable. All stigmas began opening in 30 minutes. In 90 minutes, all were fully open.

Ten stigmas of Catalpa, while the flowers were still on the tree, were covered with wheat flour and made to close. The temperature was 27°, and the air fairly moist, but the sky clear. Cheesecloth bags were drawn over the panicles. After 3 hours, 5 stigmas were still closed, and the other 5 were open slightly from the tips of the lobes to their insertion. The next observation was made the next morning, 18 hours after the stigmas were closed; all but one stigma were open. Three stigmas, as controls, were closed but given no flour at the same time as the last 10, and were otherwise given the same treatment. All three were wide open when observed two and three fourths hours after closing, and still open 18 hours after closing. Eighteen flowers of Catalpa were plucked, placed in a damp chamber and brought into the house, where the temperature was the same as outdoors, 23°; there had been showers, and the air was fairly moist. Eleven stigmas were given a thin coating of wheat flour, and 7 stigmas a heavy coating, and all stigmas were closed in the act of applying the flour. After 150 minutes, 7 were closed, 2 were open, and 2 were one third open of those receiving but a thin cover of flour Of the 7 stigmas receiving a thick cover of flour, 4 were closed and 3 were open 30°, 150 minutes after the first closing. Six hours after the flour was applied, of the 11 receiving little flour, 6 were closed, and 5 were open; of those receiving much flour, 4 were closed and 3 were open. Seventeen hours after the flour was applied, of the 11 receiving little flour, 5 were closed, 5 were wide open, and one was open 45°; of the 7 receiving much flour, 4 were closed, and 3 were open 90° to 200°.

Sixteen stigmas of *Tecoma radicans* were given dry wheat flour, and closed, the blossoms being kept in the house, standing in water in beakers. The behavior of 7 of these stigmas was followed for 3 days. Six of them remained continuously closed, and one opened during the night, at some time within 12 hours of closing. Of 7 other stigmas, of the group of 16, 6 remained closed and one opened during 4 hours, when observations were discontinued. The other 2 stigmas, one hour after receiving flour and closing, were open 60° and 90° respectively, but were fully closed two and one half hours after receiving the flour.

That the presence of the flour had the effect of keeping the stigmas of Tecoma closed was demonstrated by the behavior of several controls used at the same time as some of the 16 stigmas whose behavior has just been followed. Five stigmas were caused to close without being given anything; all opened within an hour and remained open during the 6 hours of observation.

Mimulus glabratus var. *Jamesii*, it will be recalled, has a closed flower, and its stigmas do not remain closed after pollination. Flour was used on 10 stigmas of this species, and the results showed that flour neither causes the stigmas to close, nor does it prolong the closure if one closes the stigmas at the time the flour is applied.

From the foregoing results, the stigmas of *Mimulus glabratus* and of *Torenia fournieri*, as far as the evidence goes, seem to behave in about the same way when given flour as when given pollen. That is, neither pollen nor flour keeps the stigmas closed. The stigmas of Tecoma and of Catalpa are certainly affected by the flour in that a larger proportion remain closed if given flour than if given no flour; but the flour does not keep so many closed as does the pollen. The difference in behavior with pollen and with flour is especially marked 12 to 20 hours after the primary closing: with pollen, the stigmas are likely to remain closed; but with flour the stigmas generally open finally.

Starch. Wheat flour was heated to 80° in tap water to destroy the enzyme. It was then washed 3 times to remove the protein. The starch obtained was dried and applied to the stigmas of 9 flowers of the Catalpa. The preparation was kept in the house. After 3 hours 4 were open, 4 were closed, and one was open 30°. Twelve hours after applying the starch, all stigmas were open. Twelve other stigmas were given dry starch and all stigmas were stimulated to close. This preparation also was made in the house. After one hour, 11 stigmas were open, the other closed. This condition continued for 5 hours at least, and the next observation was made the next morning, 17 hours after the starch was applied. All 12 stigmas were then open. As a control for the last preparation, 9 stigmas were pollinated and closed. The Catalpa flowers for this test were plucked at the same time as the preceding set that were given starch, and the two groups were carried on side by side. At the end of 75 minutes and of 135 minutes, 8 were open and one closed. Five and one quarter hours after pollination and the primary closing, 5 were closed and 4 open. After 16 hours from the beginning, 6 were closed and 3 were open.

In the preceding section, Tecoma was found to keep its stigmas closed when given wheat flour. A test with starch only, using two stigmas in flowers in the house, showed the starch keeping the stigmas closed for 23 hours, when observations were discontinued.

Emery powder. Very fine emery powder was washed in strong alcohol and later in several changes of water, and then dried. Large amounts of this powder were placed in the angle of the divergent lobes of eight stigmas of *Tecoma radicans.* The flowers bearing these stigmas were brought into the laboratory at noon, and set in beakers with their lower ends in water. The emery powder was applied at 1:30 P.M. and the stigmas were immediately closed. At the same time six stigmas of flowers similarly treated were given wheat flour and closed. Observation was not made for four and one half hours, when all stigmas given emery powder were open, and all given flour were closed.

3. *Living and dead pollen, enzymes, proteins.* The foregoing results obtained with Tecoma, in the behavior of stigmas when applying emery powder compared with the behavior when applying wheat starch, and with Catalpa when using wheat flour compared with the behavior when using pollen, show that the flour exerts an influence not possessed by the emery powder, and the pollen exerts an influence not possessed by the flour. Other results have shown with Catalpa that wheat flour exerts a closing effect to a greater degree than the flour deprived of its protein. In attempting to solve some of the questions arising from these relations, one may assume that it is either the proteins or the enzymes in flour that make it more effective than starch, and that it is either the enzymes or the germination effects of pollen that make pollen more effective than flour and starch in inducing permanent closure of the stigmas.

To determine the difference in behavior of stigmas with living and with dead pollen, a quantity of pollen from recently opened anthers of Catalpa was covered with water at 88° C. The water was decanted and the pollen grains were washed in water and dried at 60°. The pollen was then placed on 12 stigmas of flowers standing in water in the house, and the stigmas closed. The weather was warm and moist. Eleven of the stigmas opened within an hour. In three hours all were open. Seventeen hours after applying the pollen, all stigmas were still open. There were no controls made at the same time as the foregoing tests, but the day before, under similar conditions, four stigmas of Catalpa were given living pollen and closed. In one hour and three quarters, two stigmas were open 30° at the tips, the other two were closed. Five and three quarter hours after pollination, all were closed. The next morning, 16 hours after pollination, all were closed. Many other tests made with living pollen show that this result obtained with four stigmas is usual, and there can be no doubt that dead pollen is much less effective than living pollen in keeping the stigmas of Catalpa closed.

To test the action of an enzyme directly, the commercial Taka-diastase was used on the stigmas of Catalpa and Torenia. Thirteen flowers of Catalpa, sitting in a little water in the house, temperature 26°, moisture medium, had the Taka-diastase powder inserted into the angle of their divergent stigma lobes by using a little wooden rod whose tip was sharpened to a wedge. The stigmas closed immediately. After one hour, all were closed except one, which was well open. The same condition continued for 6 hours, the time of the last observation of the day. The next morning, 18 hours after the closing of the stigmas, 5 stigmas were open and 8 were closed. In another test, 10 stigmas of Catalpa, flowers treated as was the last set were given a mixture of one fourth Taka-diastase and three fourths starch. All stigmas remained closed for the rest of the day's observations, or for 6 hours. The next morning, 18 hours after the stigmas closed, 5 were closed and 5 were open.

At the same time that the foregoing experiments were being conducted, 11 flowers of Catalpa, treated as were the other flowers, had pollen placed on their stigmas, and the stigmas closed. After 2 hours, 6 were open and 5 closed. An hour later, 4 were open and 7 closed. The next morning, 13 hours after pollination, 4 were open and 7 closed; but one that had been open the evening before was now closed, and one that had been closed was now open. This experiment was made as a control for the preceding. It here shows the diastase acting as well as the pollen in keeping the stigmas closed. The Taka-diastase is known to contain both diastase and hemicellulase.

In an attempt to ascertain whether it was the enzyme content or the protein constitution of the Taka-diastase that kept the stigmas closed, the enzyme was destroyed by heating some of the powder in water at 80° and subsequently evaporating at a temperature of 50°. The resultant horny mass was powdered, but found to kill some of the stigmas when applied to them. Some of this powder was, therefore, mixed with three times its volume of corn starch, and the mixture was applied to the stigmas of 13 flowers of Catalpa. The flowers were kept with their bases sitting in water in the house; the experiment was made the day following the preceding one. One hour after applying the mixture and closing the stigmas, all were still closed. Two hours after the beginning, all were closed; 5 hours after the beginning, 6 were open and 7 closed; 9 hours after the beginning, 10 were open and 3 closed; 11 hours after the beginning, 10 were open and 3 closed;

the next morning, or 20 hours after the first closing, 10 were open and 3 closed. At the time of the last record, 2 that had been open had closed, and 2 that had been closed had opened.

Several potted plants of *Torenia fournieri* that were kept outdoors under fine nets were used in testing the action of Taka-diastase. On 16 stigmas was placed a mixture of one fourth of the diastase whose enzyme had been destroyed by heating and three fourths corn starch. The mixed powder was dry when applied, and all stigmas were closed in the application. After one hour 10 were open and 6 closed; after 2 hours, 11 were open and 5 closed; after 4 hours, 12 open and 4 closed. The next morning, 14 hours after the first closing, 13 were open and 3 closed. Sixteen other stigmas, used at the same time as the preceding 16 and on the same plants, were given a mixture of one fourth normal Taka-diastase and three fourths corn starch. After one hour, 5 were open and 11 closed; after 2 hours, 6 were open and 10 closed; after 4 hours, 7 were open and 9 closed. The next morning, 14 hours after the first closing, 12 were open and 4 closed.

The foregoing tests with Torenia give a very good indication that the presence of the enzyme has an effect in keeping the stigmas closed longer than the same substance when the enzyme has been destroyed; but, after the lapse of 14 hours, about as large a proportion of stigmas opened with enzyme as with the de-enzymized material.

Eight stigmas of Torenia on a plant outdoors under a fine net were given commercial casein and closed. The weather was sunny and fairly dry. All stigmas opened within 20 to 40 minutes of closing. Observations were not made to see how much earlier they may have opened. These stigmas were repeatedly closed by stimulating after the first closing, but all re-opened as often as they were artificially closed. Observations continued for 17 hours.

Twelve stigmas of Torenia, weather cloudy and moist, were given a mixture of one fourth Witte's peptone and three fourths corn starch. All opened promptly and remained open for 4 hours at least, when observation was ended.

III. Summary

In all, 25 species and varieties of plants have been reported, 4 of them for the first time in the present paper, with sensitive stigmas. These 25 plants are included in 4 families. The stigmas are composed of two tongue-shaped lobes of equal or unequal size which diverge 90° to nearly 360° when ready for pollination. The receptive region for pollen is the inner or apposed surfaces of the two lobes, except for Utricularia in which only the lower lobe receives the pollen.

The natural stimulus for the stigmas is the pressure of the body of the visiting insect or other animal, and the response consists in the closing together of the two lobes, so that the two lobes, except in the case of Utricularia, are in contact over the most or the whole of their inner surfaces. It requires an appreciable pressure to call forth a response, and pollen may be artificially applied to the stigmas without causing closing. Besides pressure, the stigmas will respond to an electric current, stigmas of some species to various vapors perhaps, and the stigmas of *Catalpa bignonioides* and *Mimulus glabratus* var. *Jamesii* to crushing of the style, or to pinching of the style.

As far as good evidence goes, the most of the species so far reported show a sensitiveness to pressure on the inner surface only. In my own work, *Catalpa bignonioides* showed itself sensitive on both the outer and the inner surfaces of the stigma lobes, though more sensitive on the inner surface. The conduction of a stimulus from one stigma lobe to another has been determined positively for only the three species so reported by Oliver—*Martynia lutea, M. proboscidea*, and *Mimulus cardinalis.* On the other hand, Lloyd found the stigma of *Diplacus glutinosus*, and my study showed the stigmas of *Catalpa bignonioides*, *Mimulus punctatus*, and *Torenia fournieri* not transmitting the stimulus from one lobe to the other.

As to the degree of relative sensitiveness, or the speed of response, in the various species, little that is certain can be said. It is certain that *Digitalis purpurea* is the slowest of all reported; but the stigma of this species does not carry out an effective closing, and it should not be considered in the same class as the others. Most of the stigmas whose time of response has been reported have shown a complete closing within 10 seconds of the time of stimulation. Stigmas, reported by Heckel as the slowest in response, I have found the quickest, namely, those of Torenia closing completely in 2 seconds, and those of Tecoma in 3 seconds after stimulation. But the responses of all stigmas vary so much with temperature and other conditions that a statement of relative sensitiveness is not now possible.

The obvious benefit to the plant of the closure of the stigma is its aid in securing cross-pollination. As the stigmas are generally in such a position as to be touched by the insect, or other visitor, before the pollen of the same flower is encountered, and as the stigmatic surface is in a few seconds after touching turned so as to be out of reach of the flower visitor as he withdraws, self-pollination can be possible only in those rare cases of very slowly acting stigmas or unusually placed stigmas.

The present study has shown that the phenomenon of stigmas opening soon after pollination is more general than hitherto supposed. We knew from the work of Lloyd that *Diplacus glutinosus* regularly opened its stigma after closing at the time of pollination. The study here reported shows that the stigmas of *Utricularia vulgaris* and *Mimulus glabratus* also always open after pollination, that the stigmas of *Torenia fournieri* and *Catalpa bignonioides* sometimes open in not very dry air, and half of them, or more, will open in moist weather. Other species have shown stigmas opening in moist air after being closed at the time of pollination.

After the primary closing at the time of pollination, the stigmas that open close again 2 to 10 hours after the opening, unless the air is very moist. The significance of this secondary closing is well indicated by the fact that pollen will not germinate on the open stigma unless the air is well nigh saturated. *Utricularia vulgaris* and *Mimulus glabratus* var. *Jamesii*, having closed flowers, do not keep their stigmas closed after closing at the time of pollination, but the pollen germinates on the open stigma lobes. Except for the two species just mentioned, the germination of the pollen on the stigmas is secured either by continuous closure of the stigmas from the time of pollination, or by a secondary closing in those cases in which the stigmas open after pollination.

As is well known, the sensitive stigmas under consideration reopen as often as closed, provided no pollen has been placed on the stigmatic surface. When pollen is placed on the stigma and the lobes are closed, the stigma may open within an hour, as it regularly does in Diplacus, or it may never open again, as is usual in Tecoma and frequent in several other species. Also the pollen may be artificially placed on the stigma so gently as not to cause closing. In this last case, the stigma remains open for two to ten hours, and then closes unless the air is very moist. In any event, the stigma is usually closed 10 hours after pollination. By what agency is the primary closing maintained, and the secondary closing caused? It has been suggested by previous writers that the withdrawal of water by the pollen is the cause of the closing, and that the phenomenon is purely physical. Numbers of experiments have been reported in this paper which seem to me to indicate that the continued closure of the stigmas is more than the simple abstraction of water. Inorganic powder, like emery flour, is not effective at all in keeping the stigmas closed, though by capillarity it should withdraw water from the closed stigma. Pollen killed in hot water and dried does not keep the stigmas closed, though it retards somewhat their opening. Wet pollen and dry pollen keep the stigmas closed equally well. Flour and starch keep the stigmas of Tecoma closed about as well as does living pollen, but the stigmas of Torenia and Catalpa open finally when given flour or starch. The enzyme known as Taka-diastase, either in pure powder or mixed with starch, causes the stigmas of Torenia and Catalpa to remain closed for 3 to 10 hours, but nearly all the stigmas finally open. The Taka-diastase, with its enzyme destroyed by hot water, then evaporated, powdered, mixed with starch, and placed on stigmas, keeps the stigmas closed a shorter time than does the mixture of the normal Taka-diastase and starch. Casein alone does not affect the closure of the stigmas of Torenia, nor does a mixture of peptone and starch. This statement of results with different species shows how impossible it is to generalize by the study of a single species, and it might be that the behavior of several similarly acting species would not lead to a correct interpretation applicable to all.

The one statement which seems possible to make is that, with the exception of *Tecoma radicans*, none of the species reported in this paper keep their stigmas closed as long with any other substance tried as with living pollen. The behavior of the stigmas of the various species tested in the present study indicates the possibility of an enzyme, or other chemical, in the pollen and Taka-diastase as the agent in maintaining closure. Starch alone is certainly effective in prolonging the closure; so that an enzyme, or similar chemical, need not be credited alone with influence on maintaining closure. It is difficult to conceive of the pollen maintaining closure or causing closure for days merely through the abstraction of water. Is there any reason why the stigmatic cells should not replenish their lost water by calling on the water supply in the style and parts below? Although the stigmas of Catalpa usually close at the beginning of wilting of the flower, the stigmas of other species do not close on wilting of the flower, and the stigmas remain open even when withering. A loss of water by the cells of the stigma, therefore, does not necessarily require the lobes to close. It is true that if pollen, flour, and starch take water from the stigma, they take it probably from one side; but we do not know that these objects certainly take more water from the cells than the cells can recover. It does not seem likely either that the absorbing objects continue their water abstraction for hours. It would seem probable that the presence of absorbing material on the stigmatic surface reduces the resistance of the protoplasm to filtration, and that this loss of resistance is gradually recovered except in the case of germinating pollen. Such an increase and maintenance of permeability, even though the effect comes slowly, may be a sensitive reaction instead of what is usually called a purely physical reaction, as has been suggested.

List of Plants Reported as Having Sensitive Stigmas, with the Authors' Names

Several authors have reported plants, giving the genus only; such are not listed here except in two cases in which no species of the genus has been published. Several of the plants listed are horticultural varieties; but I have given the names as published by the authors of the papers.

SCROPHULARIACEAE

Mimulus guttatus	Kabsch
M. moschatus	Henderson
M. luteus	Henderson
M. roseus	Henderson
M. cardinalis	Henderson
M. tillingii	Burck
M. hybridus	Burck
M. luteus L. var. *punctatus*	Newcombe
M. glabratus KBK. var. *Jamesii* T. & G.	Newcombe
Torenia Fournieri	Burck
Rehmannia sp.	Kerner
Digitalis purpurea L.	Newcombe

MARTYNIACEAE	
Martynia lutea	Heckel
M. proboscidea	Heckel
M. fragrans	Burck
M. formosa	Burck
Diplacus puniceus	Henderson
D. glutinosus Nutt.	Lloyd
BIGNONIACEAE	
Bignonia sp.	Delpino
Tecoma radicans	Heckel
T. grandiflora	Heckel
Amphicome arguta	Heckel
Incarvillea Delavayi	Burck
Catalpa bignonioides Walt.	Newcombe
C. syringifolia	Heckel
LENTIBULARIACEAE	
Utricularia vulgaris	Hildebrand

UNIVERSITY OF MICHIGAN,
ANN ARBOR

BIBLIOGRAPHY

1. **Henderson, J.** On the structure of the stigma in Mimulus and Diplacus. Ann. Nat. Hist. 6: 51. 1841.
2. **Kabsch, W.** Anatomische und physiologische Untersuchungen über einige Bewegungserscheinungen im Pflanzenreiche. Bot. Zeit. **19**: 36. 1861.
3. **Hildebrand, F.** Federigo Delpino's Beobachtungen über die Bestäubungsvorrichtungen bei den Phanerogamen. Bot. Zeit. **25**: 28. 1867.
4. **Müller, F.** Befruchtungsversuche an Cipó alho (Bignonia). Bot. Zeit. **26**: 625. 1868.
5. **Hildebrand, F.** Weitere Beobachtungen über die Bestäubungsverhältnisse an Blüthen. *Utricularia vulgaris.* Bot. Zeit. **27**: 505. 1869.
6. **Batalin, A.** Beobachtungen über die Bestäubung einiger Pflanzen. Bot. Zeit. **28**: 53. 1870.
7. **Müller, H.** Die Befruchtung der Blumen durch Insekten. Leipzig, 1873.
8. **Heckel, E.** Du mouvement dans les stigmates bilabiés des Scrophularinées, des Bignoniacées et des Sésamées. Compt. Rend. Acad. Sci. Paris **79**: 702. 1874.
9. **Darwin, C.** The effects of cross and self-fertilisation in the vegetable kingdom. London, 1876.
10. **Oliver, F. W.** Ueber Fortleitung des Reizes bei reizbaren Narben. Ber. Deutsch. Bot. Ges. **5**: 162. 1887.
11. **Correns, C.** Ueber die Abhängigkeit der Reizerscheinungen höherer Pflanzen von der Gegenwart freien Stickstoffes. Flora **75**: 87. 1892.
12. **Burck, W.** On the irritable stigmas of *Torenia Fournieri* and *Mimulus luteus* and on the means to prevent the germination of foreign pollen on the stigma. Kon. Akad. Wetensch. Amsterdam, Proc. Sect. Sci. **4**: 184. 1902.
13. **Elrod, M. N.** Botanical notes. Proc. Ind. Acad. Sci. 1903: 119. 1904.
14. **Lloyd, F. E.** Certain phases of the behavior of the stigma-lips in *Diplacus glutinosus* Nutt. Plant World **14**: 257. 1911.
15. **Brown, W. H.** The phenomenon of fatigue in the stigma of Martynia. Philip. Jour. Sci. C. Bot. **8**: 197. 1913.

THE RELATION OF AGE TO SIZE IN CERTAIN ROOT CELLS AND IN VEIN-ISLETS OF THE LEAVES OF *SALIX NIGRA* MARSH.

Marjorie A. Tellefsen

(Received for publication June 23, 1921)

Introduction

Careful microscopic studies of the effect of age on plant structure are few. Benedict in 1915 found that structural changes in Vitis and in certain other plants are closely correlated with the age of those plants. He states that the vein-islets in the leaves of *Vitis vulpina* become smaller as the vine becomes older. This decrease in size is due to the encroachment of vascular tissue. Ensign in 1919 found that the size of the vein-islets of Citrus leaves is closely correlated with the maturity of those leaves. From the most immature to the fully matured leaves of seedlings there is a gradual increase in the size of vein-islets.

Since roots of cuttings develop from meristematic cells, the writer decided to see if the age of the trees from which cuttings are made affects the size of the cells in the roots developed from them. It was also desired that further investigations be carried on with leaf tissue, to determine in particular if the age of a tree is in any way correlated with the size of vein-islets in the leaves of that tree. A woody perennial, *Salix nigra* Marsh., was chosen for the investigation.

The black willow was selected, first, because it is abundant in the region of Cincinnati, Ohio, where the investigations were carried on, and second, because of the comparative ease with which specialized tissues, *i.e.*, roots, etc., will develop from the meristematic tissue of cuttings under laboratory conditions, thus rendering them available for study. Furthermore, the black willow was chosen because of its great ability to reproduce vegetatively as well as sexually.

Because the relation of reproductive methods to rejuvenescence has not been fully determined, the problem has lost none of its interest. Sexual reproduction is generally believed to effect complete rejuvenescence of protoplasm. The effects of asexual propagation are still discussed.

Among the lower forms of life many plants and animals reproduce asexually for a considerable length of time, and for these organisms this type of propagation seems to effect rejuvenescence. Some of the unicellular forms are known to reproduce in no other way. As organisms become more and more complex, sexual reproduction appears and in many forms entirely replaces asexual propagation, while in other species both sexual and asexual reproduction occur.

Because sexual reproduction causes rejuvenescence, the efficiency of vegetative propagation in these more complex organisms has become a subject for investigation. The question is especially pertinent because many of the higher plants, especially cultivated forms, are propagated extensively by means of cuttings. If vegetative reproduction does not effectively rejuvenate protoplasm in these complex forms, plants grown from cuttings are as old as the plant from which the cutting was taken. The direct bearing of this question on the commercial propagation of certain cultivated plants is evident. *Salix nigra* Marsh., the species selected for the present investigation, is one in which the power of vegetative propagation is well developed, and one in which sexual reproduction occurs regularly. In such a species any relation exhibited between age and cell structure is particularly significant.

Collection and Care of Cuttings

During the fall of 1917 and the spring of 1918, cuttings were made from trees of *Salix nigra*. They were tagged, numbered, and taken to the laboratory where they were placed in warm water over night. The following day the cuttings were put in jars of tap water. Fresh water was supplied every few days. About a month later the cuttings were transferred to a galvanized iron tank of running water. A slatted arrangement made of wood floated on the water and served to hold the cuttings up and to keep them from too close contact with each other. A correct balance could not be determined between the required intake of water for aëration and the requisite temperature for root formation, so that water molds developed and killed the cuttings. However, a few roots were formed, and these were killed and imbedded for study.

During the fall of 1918 and the early spring of 1919, cuttings were made from branches of recent growth of trees of *Salix nigra*. The age of each tree was determined roughly by measuring the diameter of the main trunk of the tree six inches above the ground. The latter distance was arbitrarily chosen so that measurements would be taken a uniform distance above the ground. The trees were then classified according to their diameter. Three groups were differentiated and arbitrarily distinguished by the letters A, B, and C. Trees less than 2½ inches in diameter were considered to be in group A, those 4 to 6 inches in diameter in group B, and those 8 inches or more in diameter in group C.

Cuttings were tagged and taken to the laboratory, where they were placed in a warm bath (38°–40° C.) for two hours to stimulate protoplasmic activity. When removed from the bath, some were placed in the tank of running water. The majority of the cuttings, however, were put in glass jars which were then placed either in Wardian cases in diffuse sunlight or on window sills supplied with bottom heat from radiators. The water in the jars was renewed as needed and entirely changed about once a month.

Benedict (1915) in writing of senile changes in physiological activities of plants says that "the most obvious characteristic is a decrease in rate of growth." McFarland mentions in a recent test that the regenerative power appears to be greater in proportion to the youth of an animal. The present writer found that cuttings from younger trees rooted in less time than those from older trees. Leaves appeared on cuttings of younger trees before they did on those of older trees.

When roots appeared on the cuttings, they were allowed to grow until about 2½ to 3 inches long. Then sections were cut from roots of the several cuttings 2 inches back from the root tip and put into Flemming's strong solution. The sections were taken 2 inches back from the root tip in order to find differentiation of the ground meristem.

After the usual killing, washing, and dehydration, the pieces of roots were imbedded in paraffin, and both longitudinal and cross sections were cut six microns in thickness. The sections were stained in safranin and Mayer's haemalum, or in safranin and light green. After being stained, the sections were mounted in balsam.

Measurement of Root Cells

When the prepared root sections were examined, it was found that large air spaces had developed in the cortical tissue. The number of these air spaces was found not to be constant, but varying from three to five, four being the common number. This provision for aëration gave the cross sections a decided three-, four-, or five-rayed appearance, the number of xylem strands coinciding in each case with the number of large air spaces.

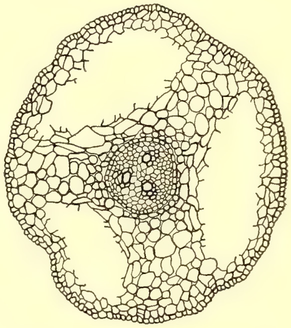


Fig. 1. Cross section of root of *Salix nigra* Marsh., showing three large air spaces and a three-rayed condition of the cortex. × 90.

Somewhat similar conditions have been reported for other plants. In 1888 Scott and Wager recorded the fact that the primary cortex of floating roots of *Sesbania aculeata* Pers. consists of rounded cells among which are very large lacunae filled with air.

Lily Batten (1918) mentions the fact that cortical root cells of *Epilobium hirsutum* are very loosely packed in young roots and that large air spaces occur in the roots of plants grown under very moist conditions.

Ada Hayden (1919) found cortical air spaces in roots of prairie plants studied.

In 1913, Norris planted *Zea Mays* in various media and examined the structure of the roots. He found that the medium used influenced root structure, particularly that of the cortex. Air spaces appeared in those roots whose surrounding medium afforded a scanty air supply. Norris reported that the cortex of roots growing in water is of a very flimsy and indefinite nature and is apt to fall to pieces when an attempt is made to section the roots. For roots grown in sawdust he reports that air spaces occur throughout and appear to be formed by the breaking down of groups of cells, the cell walls being in many cases not completely broken and stretching across the air spaces.

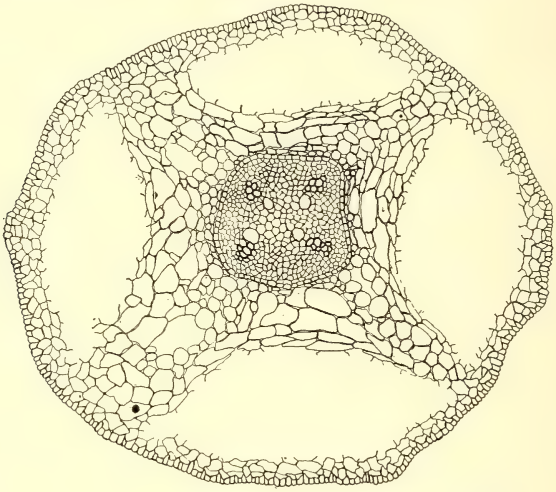


FIG. 2. Cross section of root of *Salix nigra*, showing four large air spaces and a four-rayed condition of the cortex. × 90.

The conditions found in *Salix nigra* correspond to those described by Norris for *Zea Mays.* The cortical root tissue of Salix appears very loose, and, as has been stated, the air spaces are well developed. Remnants of cell walls stretching out into these air spaces lead the writer to conclude that this provision for aëration results from the breaking down of cortical cells.

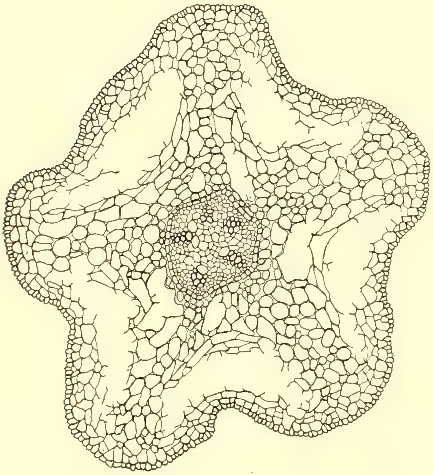


FIG. 3. Cross section of root of *Salix nigra,* showing five large air spaces and a five-rayed condition of the cortex. × 90.

When roots were removed from the cuttings to be placed in killing fluid, these air spaces appeared as continuous air passages parallel with the long axes of the roots.

The accompanying figures will serve to illustrate the kind of material used in determining the size of cells.

Five cells were selected from each tissue visible in the cross sections of about one hundred roots. The cells were chosen at random save those of the cortex. These latter were selected from the spherical cells within the cortical rays. Measurements were made in the same manner in each case. The cells were measured first through their tangential diameter and then through their radial diameter. The thickness of the cell walls was measured. All measurements were recorded in microns.

Because of the large amount of aëration tissue present, there was obvious difficulty in securing complete longitudinal sections of the roots. However, five cells were selected from the epidermis and five from the cortex of each of about thirty roots. In each case the first measurement was taken through the center of the long axis of the cell; the second measurement was made through the center of the short axis of the cell. Data were recorded in the same manner as for the cross sections.

Table 1. *Measurements in Cross Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra Marsh., Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	$\frac{1}{4}$	12.94 x 17.26
302	$\frac{3}{16}$	11.28 x 13.61
306	$\frac{3}{16}$	13.61 x 14.94
308	$\frac{1}{8}$	12.60 x 11.28
318	$\frac{3}{16}$	11.95 x 10.29
321	$\frac{3}{16}$	12.61 x 12.28
340	—1	9.62 x 10.62
343	—1	10.29 x 12.28
357	—1	11.95 x 12.26
364	—1	10.62 x 9.29
401	2	10.29 x 10.28
606	2	8.96 x 12.28
608	2	13.28 x 11.95
616	2	9.29 x 9.29
636	$1\frac{1}{2}$	13.28 x 13.94
637	2	14.44 x 18.93
638	2	9.96 x 13.77
707	2	10.79 x 20.25
710	1	8.95 x 13.01
713	1	11.62 x 13.08
714	$1\frac{1}{2}$	10.78 x 10.95
715	2	10.78 x 16.27
716	1	8.96 x 13.44
717	2	12.28 x 13.01
719	$1\frac{1}{2}$	11.62 x 13.94
720	$1\frac{1}{2}$–2	10.29 x 13.77
724	2	11.78 x 14.60
733	1	12.39 x 12.28
734	1	11.62 x 13.61
735	2	11.95 x 15.27
737	1	9.96 x 12.82
746	1	11.22 x 15.27
Average		11.31 x 13.31

TABLE 2. *Measurements in Cross Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	14.24 x 9.25
62	4	8.95 x 10.95
68	4	10.95 x 15.93
167	4½	11.78 x 10.29
330	5	11.78 x 12.28
405	4	12.69 x 14.60
601	6	10.95 x 11.62
609	4	11.95 x 11.62
612	5	9.79 x 13.11
617	4	8.30 x 12.94
621	5	11.28 x 13.92
623	6	9.62 x 12.28
627	6	10.45 x 10.95
633	4	11.28 x 14.27
635	5	12.28 x 16.93
639	4	8.30 x 10.62
641	6	11.62 x 17.92
642	5	12.61 x 13.77
700	5	11.62 x 15.27
706	6	13.94 x 16.93
Average		11.21 x 13.27

TABLE 3. *Measurements in Cross Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	13.41 x 11.95
44	10	9.62 x 13.94
46	18	12.57 x 16.50
48	10	11.96 x 12.28
151	12	11.62 x 14.92
326	13	10.06 x 15.75
327	13	9.62 x 10.55
328	13	11.41 x 14.94
400	10+	10.29 x 14.81
404	15	7.97 x 10.61
600	8–10	9.62 x 13.55
602	20	10.46 x 13.61
604	10	13.12 x 15.07
605	8	9.13 x 10.62
611	8	10.65 x 10.79
620	8	12.28 x 15.27
624	10+	9.63 x 9.63
625	10+	9.92 x 11.79
628	8	10.13 x 11.62
629	8	9.79 x 11.29
630	10	11.95 x 10.27
631	8–10	11.45 x 12.96
632	10+	12.55 x 13.11
634	10	9.62 x 13.28
643	10	10.79 x 14.94
702	10+	10.29 x 14.61
703	10+	14.44 x 11.79
704	10+	11.79 x 13.45
705	10+	13.44 x 15.10
708	8	11.62 x 15.27
Average		10.04 x 13.14

TABLE 4. *Measurements in Longitudinal Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	$\frac{1}{4}$	74.70 x 11.28
302	$\frac{3}{16}$	114.87 x 8.63
308	$\frac{1}{8}$	58.10 x 9.96
707	2	82.21 x 12.95
710	1	124.25 x 13.28
713	1	68.06 x 10.95
714	1$\frac{1}{2}$	79.45 x 8.68
715	2	101.92 x 11.28
716	1	75.03 x 11.95
717	2	108.23 x 10.12
719	1$\frac{1}{2}$	91.96 x 9.96
733	1	93.62 x 13.28
746	1	86.20 x 8.30
Average		89.12 x 10.81

TABLE 5. *Measurements in Longitudinal Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	63.36 x 10.72
62	4	55.44 x 8.30
68	4	91.63 x 13.94
Average		70.14 x 10.98

TABLE 6. *Measurements in Longitudinal Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
46	18	75.36 x 9.29
48	10	37.85 x 8.63
91	8–10	44.08 x 14.60
600	8–10	74.70 x 9.96
702	10+	101.26 x 10.63
703	10+	83.83 x 12.45
704	10+	109.56 x 9.63
729	10+	93.50 x 15.94
Average		77.51 x 11.39

TABLE 7. *Measurements in Cross Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	$\frac{1}{4}$	20.91 x 24.56
302	$\frac{3}{16}$	27.64 x 25.31
306	$\frac{3}{16}$	25.89 x 26.89
308	$\frac{1}{8}$	23.57 x 25.89
318	$\frac{3}{16}$	27.55 x 29.71
321	$\frac{3}{16}$	22.24 x 23.90
340	—1	28.88 x 27.88
343	—1	26.22 x 22.57
357	—1	27.88 x 26.91
364	—1	29.21 x 22.90
606	2	23.24 x 22.57
608	2	25.23 x 23.57
616	2	24.56 x 26.89
636	1$\frac{1}{2}$	33.21 x 29.21
637	2	36.02 x 37.82
638	2	32.20 x 31.54
640	1	40.16 x 36.18
707	2	24.90 x 23.86
710	1	28.55 x 29.21
713	1	38.53 x 37.05
714	1$\frac{1}{2}$	36.54 x 41.16
715	2	32.87 x 33.99
716	1	27.72 x 33.86
717	2	30.54 x 27.68
719	1$\frac{1}{2}$	28.22 x 33.53
720	1$\frac{1}{2}$–2	24.90 x 28.81
724	2	32.87 x 34.86
733	1	35.85 x 38.84
734	1	33.86 x 35.52
735	2	31.87 x 34.19
737	1	31.87 x 33.86
746	1	31.54 x 40.17
Average		29.57 x 30.02

TABLE 8. *Measurements in Cross Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	16.42 x 18.58
62	4	26.56 x 28.22
68	4	21.58 x 25.56
167	4$\frac{1}{2}$	29.71 x 22.72
330	5	24.23 x 29.21
405	4	26.89 x 24.56
601	6	34.19 x 31.20
609	4	29.54 x 29.54
612	5	33.86 x 30.71
617	4	26.56 x 24.07
621	5	33.53 x 29.55
623	6	28.67 x 29.38
627	6	30.54 x 32.53
633	4	36.85 x 38.78
635	5	33.53 x 36.52
639	4	29.05 x 27.37
641	6	28.88 x 33.53
642	5	31.91 x 30.87
700	5	32.03 x 33.03
706	6	30.87 x 30.87
Average		29.92 x 29.36

TABLE 9. *Measurements in Cross Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	25.54 x 22.88
44	10	22.24 x 30.87
46	18	27.22 x 28.22
48	10	31.54 x 34.52
326	13	21.91 x 26.56
327	13	27.55 x 26.56
328	13	28.85 x 27.77
400	10+	24.57 x 27.55
404	15	25.89 x 29.21
602	20	28.82 x 27.68
604	10	31.87 x 33.86
605	8	22.91 x 25.23
611	8	29.88 x 37.18
620	8	28.38 x 31.04
624	10+	30.21 x 30.54
625	10+	28.12 x 30.88
628	8	21.25 x 22.91
629	8	26.56 x 26.89
630	10	24.90 x 27.56
631	8–10	34.24 x 26.84
632	10+	30.54 x 26.89
634	10	20.24 x 20.58
643	10	32.87 x 30.54
702	10+	33.54 x 34.86
703	10+	28.72 x 29.72
704	10+	29.22 x 27.56
705	10+	34.20 x 34.87
708	8	32.20 x 36.85
Average		27.28 x 29.16

TABLE 10. *Measurements in Longitudinal Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	$\frac{1}{4}$	61.08 x 13.94
302	$\frac{3}{16}$	43.49 x 12.28
308	$\frac{1}{8}$	64.74 x 29.54
707	2	150.72 x 23.57
710	1	148.50 x 22.37
714	$1\frac{1}{2}$	80.88 x 29.55
715	2	112.21 x 17.92
716	1	86.98 x 17.43
717	2	120.35 x 23.24
719	$1\frac{1}{2}$	101.59 x 32.20
733	1	66.98 x 20.58
746	1	112.21 x 27.69
Average		95.81 x 22.52

TABLE 11. *Measurements in Longitudinal Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	70.13 x 14.59
62	4	82.33 x 15.27
68	4	95.94 x 30.21
Average		82.80 x 20.02

TABLE 12. *Measurements in Longitudinal Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
46	18	48.14 x 9.62
48	10	56.44 x 14.27
91	8–10	81.65 x 18.60
154	15	66.40 x 20.91
600	8–10	70.71 x 20.58
702	10+	117.86 x 23.24
703	10+	108.73 x 21.58
704	10+	112.88 x 17.60
Average		82.85 x 18.30

TABLE 13. *Measurements in Cross Section of Endodermal Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	¼	15.93 x 10.29
306	3/16	10.95 x 11.48
308	⅛	8.30 x 9.29
318	3/16	10.27 x 8.96
321	3/16	11.62 x 9.62
343	−1	10.95 x 7.96
357	−1	15.47 x 8.30
364	−1	14.27 x 11.62
608	2	9.96 x 8.46
636	1½	9.96 x 10.95
637	2	12.95 x 11.62
640	1	10.62 x 8.13
710	1	9.09 x 8.63
713	1	12.77 x 8.96
714	1½	12.11 x 8.96
715	2	10.95 x 8.13
716	1	10.79 x 9.62
719	1½	11.62 x 9.62
724	2	10.95 x 8.63
733	1	11.62 x 10.29
735	2	10.12 x 10.62
737	1	9.96 x 6.37
746	1	9.98 x 8.13
Average		11.31 x 9.33

TABLE 14. *Measurements in Cross Section of Endodermal Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	9.96 x 7.12
62	4	11.29 x 9.29
68	4	9.96 x 12.28
167	4½	9.79 x 8.96
330	5	8.63 x 9.29
405	4	11.12 x 10.62
601	6	11.95 x 9.62
609	4	8.96 x 7.96
612	5	9.29 x 8.30
617	4	8.50 x 7.96
621	5	9.29 x 9.29
623	6	11.95 x 8.30
627	6	11.95 x 6.97
633	4	9.59 x 10.19
635	5	8.16 x 10.95
639	4	11.12 x 8.46
641	6	10.95 x 8.63
700	5	10.95 x 11.28
706	6	12.61 x 6.38
Average		10.31 x 9.04

Table 15. *Measurements in Cross Section of Endodermal Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	11.45 x 9.62
44	10	11.95 x 9.96
326	13	14.11 x 10.97
327	13	7.74 x 8.96
328	13	10.62 x 7.77
400	10+	9.96 x 14.77
404	15	8.61 x 7.61
602	20	9.96 x 9.15
611	8	8.79 x 9.29
624	10+	11.79 x 8.30
625	10+	8.80 x 9.79
628	8	9.63 x 12.28
630	10	9.96 x 9.13
631	8–10	8.80 x 11.29
632	10+	11.79 x 8.80
634	10	10.96 x 9.30
643	10	8.96 x 9.29
704	10+	11.95 x 9.30
Average		10.32 x 9.64

Table 16. *Measurements in Cross Section of Phloem Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	$\frac{1}{4}$	9.96 x 7.63
306	$\frac{3}{16}$	6.97 x 5.97
308	$\frac{1}{8}$	6.80 x 6.97
321	$\frac{3}{16}$	9.62 x 6.97
340	−1	6.64 x 7.70
343	−1	5.91 x 6.64
364	−1	7.63 x 5.97
606	2	7.80 x 7.63
608	2	7.96 x 7.63
636	$1\frac{1}{2}$	8.04 x 7.63
640	1	7.64 x 7.97
733	1	7.96 x 6.97
Average		7.82 x 7.14

Table 17. *Measurements in Cross Section of Phloem Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	6.76 x 6.40
62	4	5.64 x 6.64
68	4	6.30 x 7.63
405	4	7.70 x 7.63
609	4	7.96 x 7.63
633	4	6.97 x 7.13
635	5	8.07 x 7.96
641	6	6.68 x 7.63
642	5	8.13 x 7.30
700	5	6.30 x 6.30
Average		7.05 x 7.22

TABLE 18. *Measurements in Cross Section of Phloem Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	8.46 x 7.07
44	10	7.30 x 8.30
48	10	8.76 x 7.63
326	13	6.97 x 9.03
605	8	6.30 x 8.30
624	10+	6.64 x 7.30
628	8	7.49 x 6.97
630	10	5.81 x 7.64
631	8–10	8.01 x 7.47
632	10+	5.98 x 6.79
634	10	7.97 x 6.64
643	10	6.64 x 5.31
Average		7.19 x 7.37

TABLE 19. *Measurements in Cross Section of Xylem Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	¼	11.62 x 14.60
302	3/16	9.13 x 8.57
306	3/16	8.30 x 6.03
308	⅛	8.30 x 7.62
318	3/16	8.30 x 10.95
321	3/16	10.62 x 8.30
340	–1	16.26 x 18.26
343	–1	8.29 x 9.96
357	–1	11.62 x 11.62
364	–1	15.27 x 15.60
401	2	8.30 x 9.95
606	2	9.29 x 11.95
608	2	14.27 x 15.27
636	1½	9.96 x 10.95
637	2	14.27 x 13.94
707	2	14.27 x 13.28
710	1	11.28 x 8.07
713	1	13.28 x 10.95
714	1½	11.62 x 13.28
715	2	9.95 x 9.44
716	1	9.15 x 11.62
717	2	8.63 x 9.62
719	1½	10.62 x 8.69
720	1½–2	7.30 x 6.64
724	2	12.08 x 12.41
733	1	13.28 x 15.93
734	1	13.61 x 13.94
735	2	9.70 x 9.62
737	1	11.25 x 11.12
Average		11.03 x 11.31

TABLE 20. *Measurements in Cross Section of Xylem Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	9.96 x 8.54
62	4	8.96 x 11.95
68	4	9.29 x 10.62
167	4½	10.95 x 11.18
330	5	9.29 x 9.29
405	4	11.62 x 12.94
601	6	7.30 x 13.28
609	4	9.62 x 10.60
612	5	11.28 x 10.95
621	5	14.94 x 14.27
623	6	8.96 x 9.46
627	6	11.28 x 6.97
633	4	12.78 x 13.61
635	5	9.40 x 12.17
639	4	15.93 x 18.92
641	6	10.79 x 10.79
642	5	10.29 x 9.62
700	5	11.95 x 11.95
706	6	10.61 x 13.28
Average		10.80 x 11.61

TABLE 21. *Measurements in Cross Section of Xylem Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	13.78 x 13.61
44	10	12.28 x 17.59
46	18	8.63 x 11.95
48	10	11.62 x 11.97
326	13	9.96 x 8.46
327	13	10.61 x 17.26
328	13	8.13 x 9.13
404	15	11.29 x 10.95
602	20	9.15 x 12.95
604	10	11.78 x 11.78
605	9	9.62 x 10.60
611	8	10.45 x 12.61
620	8	9.97 x 9.96
624	10+	11.29 x 12.95
625	10+	11.29 x 9.30
628	8	10.96 x 10.29
629	8	10.62 x 10.96
630	10	11.62 x 13.28
631	8–10	10.96 x 12.61
634	10	13.94 x 14.94
643	10	11.29 x 12.12
702	10+	13.61 x 10.63
703	10+	11.62 x 10.51
704	10+	12.28 x 9.95
705	10+	11.62 x 13.92
708	8	9.63 x 9.30
Average		11.07 x 11.90

TABLE 22. *Measurements in Cross Section of Meristematic Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
364	−1	10.29 x 10.29
733	1	9.29 x 11.28
Average		9.79 x 10.78

TABLE 23. *Measurements in Cross Section of Meristematic Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	11.74 x 11.39
62	4	10.95 x 13.28
68	4	7.30 x 9.62
617	4	8.96 x 10.45
641	6	12.28 x 12.61
Average		10.24 x 11.47

TABLE 24. *Measurements in Cross Section of Meristematic Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	10.95 x 11.62
44	10	8.63 x 9.62
46	18	12.11 x 11.28
404	15	9.63 x 9.63
602	20	7.97 x 10.95
605	8	8.63 x 11.61
620	8	9.96 x 9.29
628	8	11.95 x 11.82
631	8–10	8.30 x 15.44
643	10	10.79 x 14.94
Average		9.89 x 11.62

The measurements of the five cells of each tissue were averaged for each root measured. The averages thus obtained were averaged again according to tissues, and a grand average of cell measurements for each tissue was obtained. For example, the measurements of the five epidermal cells were averaged for each root. These averages were then added and averaged for the number of roots examined, so that the resulting grand average represented the average measurement for all the epidermal cells. A grand average was obtained for epidermal root cells from cuttings of Groups A, B, and C. These averages were then compared and tabulated as shown in tables 25 and 26. This procedure was followed for each root tissue present.

From an examination of table 25 it will be seen that the average measurements of cross sections of root cells of cuttings from trees of *Salix nigra*

TABLE 25. *Comparison of Average Measurements (in Microns) of Cross Sections of Root Cells of Cuttings of Trees of Salix nigra*

Tissue	Group A		Group B		Group C	
	Ave. Meas. of Cells	Computed Cross Sections	Ave. Meas. of Cells	Computed Cross Sections	Ave. Meas. of Cells	Computed Cross Sections
Epidermis	11.31 x 13.31	150.5361	11.21 x 13.27	148.7569	11.04 x 13.14	145.0656
Cortex	29.57 x 30.02	887.6914	29.92 x 29.36	878.4512	27.28 x 29.16	795.4848
Endodermis	11.31 x 9.33	105.5223	10.31 x 9.04	93.2024	10.32 x 9.64	99.4848
Phloem	7.82 x 7.14	55.8348	7.05 x 7.22	50.9010	7.19 x 7.37	52.9903
Xylem	11.03 x 11.31	124.7493	10.80 x 11.61	125.3880	11.07 x 11.90	131.7330
Meristem	9.79 x 10.78	105.5362	10.24 x 11.47	117.4528	9.89 x 11.62	114.9218

TABLE 26. *Comparison of Average Measurements (in Microns) of Longitudinal Sections of Root Cells of Cuttings of Trees of Salix nigra*

Tissue	Group A		Group B		Group C	
	Ave. Meas. of Cells	Computed Longitudinal Sections	Ave. Meas. of Cells	Computed Longitudinal Sections	Ave. Meas. of Cells	Computed Longitudinal Sections
Epidermis	89.12 x 10.81	963.3872	70.14 x 10.98	769.1372	77.51 x 11.39	882.8389
Cortex	95.81 x 22.52	2,157.6412	82.80 x 20.02	1,657.6560	82.85 x 18.30	1,516.1550

differ with the age of the tree from which the cuttings were taken. As the tree grows older, the epidermal and cortical cells of the roots become smaller, while the xylem and meristematic cells of these roots become larger. The endodermal and phloem cells of the roots seem to become smaller through their tangential diameters and larger through their radial diameters.

Table 26 shows that epidermal and cortical root cells seem to become shorter with the increasing age of the parent tree.

The noted increase in size of the xylem cells in cross section is particularly interesting in the light of unpublished studies by H. M. Benedict, who found that in the leaves of *Vitis vulpina* L. the amount of vascular tissue not only increases, but also that the xylem cells become larger as the vine grows older.

Concerning *Salix nigra*, one may conclude from the material examined that the age of the tree seems to affect the size of root cells of cuttings.

Collection and Care of Leaves

During the fall of 1917 and the fall of 1918, leaves were collected from about seventy black willow trees. The age was determined as stated above

Table 27. *Average Areas of Vein-islets of Leaves of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Areas of 20 Vein-islets (Sq. Mm.)
8	1½	.244
11	½	.440
16	2	.305
17	1	.440
23	1	.423
24	1	.458
25	1¼	.423
27	2	.268
29	2	.323
30	½	.354
31	1	.314
32	2	.423
37	2	.611
38	2½	.478
39	1½	.323
49	2	.578
51	1½	.458
52	2½	.440
70	¼	.550
71	1	.550
72	¼	.458
73	¼	.610
74	3/16	.550
75	3/16	.393
77	¼	.440
78	¼	.687
79	¼	.354
80	⅛	.407
81	3/16	.407
82	⅛	.440
83	3/16	.354
84	3/16	.478
85	⅛	.407
86	⅛	.478
Average		.4369

in discussing the collection of cuttings. As before, a tree less than 2½ inches in diameter was considered to be in group A, one 4 to 6 inches in diameter in group B, and trees 8 or more inches in diameter were considered to be in group C.

TABLE 28. *Average Areas of Vein-islets of Leaves of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Areas of 20 Vein-islets (Sq. Mm.)
2	4	.261
3	5	.314
6	6	.297
7	6	.261
9	5	.275
10	5	.305
18	4	.333
21	6	.239
28	5	.261
33	5	.524
36	4-5	.524
47	5	.343
57	4	.282
59	4	.289
61	4	.379
64	4	.393
65	4	.360
66	5	.379
67	5	.220
69	5	.323
Average		.3281

TABLE 29. *Average Areas of Vein-islets of Leaves of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Areas of 20 Vein-islets (Sq. Mm.)
1	8	.382
5	8	.261
12	10	.244
13	15	.275
14	12	.244
15	10	.282
19	13	.289
20	12	.323
22	8	.330
35	10	.500
Average		.3130

TABLE 30. *Comparison of Average Areas of Vein-islets of the Leaves of Salix nigra*

Average Area of Vein-islets of Leaves of Trees, Group A	.4369 sq. mm.
Average Area of Vein-islets of Leaves of Trees, Group B	.3281
Average Area of Vein-islets of Leaves of Trees, Group C	.3130

About twenty leaves of average size were picked from each tree. They were carefully placed in envelopes each bearing the number of the tree from which they were collected.

Measurement of Vein-islets

After the leaves were taken to the laboratory, they were cut transversely at their broadest point and the cut ends of the veins and veinlets were counted. The width of the leaves was measured at the place where the veinlet count was made. All data were tabulated.

The number of cut ends of veins in the twenty leaves from each tree was added and averaged to find the average number of veinlets for the entire tree. This number was then divided by the average width of the twenty leaves to obtain the average area of the vein-islets for the whole tree. This method was used for each of the seventy trees. A grand average was then made of vein-islet areas of trees in groups A, B, and C. These areas were compared and tabulated as shown in tables 27–30.

It will be seen from an examination of table 30 that the average area of vein-islets in the leaves from trees in group C is smaller than in leaves from trees in groups A and B. The average area of vein-islets of trees in group C is 71 percent smaller than that of vein-islets of trees in group A.

Summary and Conclusions

1. Cuttings from younger trees of *Salix nigra* Marsh. rooted in less time than those from older trees.

2. Leaves appeared on cuttings of younger trees before they did on those of older trees. Age seems to be correlated with a decrease in rate of growth.

3. The number of xylem strands coincides with the number of large cortical air spaces.

4. Epidermal and cortical root cells of cuttings seem to become smaller with the increasing age of the parent tree.

5. Xylem and meristematic root cells of cuttings seem to become larger as the parent tree becomes older.

6. Endodermal and phloem root cells tend to decrease in size through their tangential diameters and to increase in size through their radial diameters.

7. The age of the parent tree apparently affects the size of root cells of cuttings from that tree.

8. The average area of vein-islets in leaves from older trees is smaller than average vein-islet areas of leaves from younger trees. With the onset of senility the amount of vascular tissue seems to increase, thus reducing the average area of vein-islets.

9. Large air spaces were found in the cortical tissue of the willow roots. The number of these spaces is not constant. Four is the average number.

The writer wishes to acknowledge gratefully the help so willingly given by many friends and colleagues in the preparation of this paper. My thanks are especially due to Prof. Harris M. Benedict of the University of

Cincinnati for suggesting the problem and for his kindly and patient direction of the work.

BIBLIOGRAPHY

Batten, Lily. 1918. Observations on the ecology of *Epilobium hirsutum.* Jour. Ecol. **6**: 161–177.

Benedict, H. M. 1915. Senile changes in leaves of *Vitis vulpina* L. and certain other plants. Cornell Univ. Agr. Exp. Sta. Memoir 7.

Brotherton, W., and Bartlett, H. H. 1918. Cell measurement as an aid in the analysis of quantitative variation. Amer. Jour. Bot. **5**: 192–206.

Curtis, O. F. 1918. Stimulation of root growth in cuttings by treatment with chemical compounds. Cornell Univ. Agr. Exp. Sta. Memoir 14.

Ensign, M. R. 1919. Venation and senescence of polyembryonic Citrus plants. Amer. Jour. Bot. **6**: 311–329.

Hayden, Ada. 1919. The ecologic subterranean anatomy of some plants of a prairie province in central Iowa. Amer. Jour. Bot. **6**: 87–105.

Lamb, G. N. 1915. Willows: Their growth, use and importance. U. S. Dept. Agr. Contrib. For. Serv. Bull. 316.

Norris, F. D. M. 1913. Production of air passages in the root of *Zea Mays* by variation of the culture media. Ann. Rep. and Proc. Bristol Naturalists' Soc. IV, **3**: 134–136.

Scott, D. H., and Wager, H. 1888. On the floating-roots of *Sesbania aculeata* Pers. Annals of Bot. **1**: 307–314.

THE ORIGIN OF NEW VARIETIES OF NEPHROLEPIS BY ORTHOGENETIC SALTATION

II. REGRESSIVE VARIATION OR REVERSION FROM THE PRIMARY AND SECONDARY SPORTS OF *BOSTONIENSIS*[1]

R. C. Benedict

(Received for publication June 30, 1921)

Introduction: A Review of the Characters of the Progressive Sports

A description of the regressive variations of *Nephrolepis exaltata* var. *bostoniensis* requires some review of the characters of the progressive sports of this form.[2] The nature of these may most simply be indicated by tabulating the outstanding facts relating to them.

1. The progressive varieties so far described all have had their origin by *vegetative* sporting from *N. exaltata* var. *bostoniensis*, or its derivatives. Reproduction is by bud plants on slender stolons.

2. Over *one hundred* new varieties have appeared spontaneously at various florists' establishments in the last twenty years.

3. All these varieties have been *discontinuous*, *i.e.*, separated from the parent form by a definite break in characters—in other words, saltations or mutations. These new varieties have not intergraded by slight differences into their parent forms.

4. The evolution of these forms has been *orthogenetic*, that is, they have developed in series in which each character is successively intensified in succeeding forms. There are three such orthogenetic series:

> (*a*) Division series: *bostoniensis*, once pinnate—*Piersoni*, twice pinnate—*Barrowsi*, more completely twice pinnate—*Whitmani*, three times pinnate—*Smithi*, four times pinnate—*Craigi*, five times pinnate.
>
> (*b*) Ruffling series: *bostoniensis*, with nearly plane pinnae—*Roosevelti*, pinnae ruffled—sport of *Roosevelti*, pinnae more ruffled and lobed.
>
> (*c*) Dwarfing series: *bostoniensis*—*Scotti*—*Wagneri*, and *bostoniensis*—*Giatrasi*—sport of *Giatrasi*; the second and third forms in each case are successively smaller than *bostoniensis*.

As further evidence of orthogenesis may be cited the fact that combinations of these three types of variations may occur. Thus, dwarfing may appear in the division series and in the ruffling series, and division may appear in the dwarfing series (*cf.* chart, page 144).

---

[1] Brooklyn Botanic Garden Contributions, No. 27.

[2] For a full account, see Bull. Torrey Bot. Club **43**: 207–234. Pl. 10–15. 1916.

5. The varieties, even in a single series, are almost invariably characterized by several *associated differences.* Thus *Giatrasi,* which is half the size of *bostoniensis,* differs also in the possession of red, wiry, somewhat sinuous petioles and rachides, and by differently shaped pinnae. In only a few cases is there but one distinguishing character obvious.

6. The new varieties are *stable, i.e.,* they reproduce themselves true to type in a high percentage of cases. Occasionally in the division-series forms, reverting leaves may appear; that is, leaves with less division than is typical; and furthermore, new runner plants may develop which show a return in characters toward the parent form. The latter are uncommon, however, and, as will be indicated later, are actually new saltations or mutations. Some varieties have not as yet been known to show any variations from type.

7. The progressive varieties have been *infrequent.* If reversions are uncommon, as noted in the preceding paragraph, new progressive forms have been much more so. Of the millions of plants of the original *bostoniensis* variety grown for sale, only six or eight sports have been reported. The large number of one hundred progressive sports in twenty years has been due to two factors: to the fact that millions of these plants are grown each year and that florists are on the watch for new forms, and to the fact, also, that the coefficient of mutation has been higher in some of the derived varieties than in *bostoniensis* itself.

As a basis for the description and comparison of the reversionary forms, reference is here made to the revised chart of the relationships of the progressive varieties considered in the earlier paper. The revision consists mainly in the omission of a few forms of some uncertainty of origin and of no particular interest in connection with the present paper. *Schilleri* and *Schultheisi,* before attributed to *bostoniensis* as primary sports, are not in circulation in the trade or likely to be. *Wittboldi,* included doubtfully in the *bostoniensis* chart of the earlier paper, may almost certainly be eliminated from any such relationship. Although no plants have been obtainable from the original producer, specimens have been received from a number of sources, French, English, and American, which by reason of their exact agreement in characteristics may be considered authentic. Judged from this material, *Wittboldi* is a variety of some species other than *exaltata,* to which it was originally attributed. It probably belongs with *biserrata.* At any rate, it is of no interest in the present paper.

The position of the three- to four-pinnate *Amerpohli,* originally placed as a primary sport of *bostoniensis,* has been changed to bring it opposite varieties of a similar amount of division. No more evidence of its exact origin is available, but since this is in doubt in any event, it seems best to place it with similar forms. *Smithi* and *Craigi,* originally attributed in doubt to *Amerpohli*, have also had their position changed, and on the basis of close resemblance have been placed with the sports of *Whitmani* to which they are probably related.[3]

The Nature of Regressive Variation

It is common knowledge that some of the Boston-fern varieties as they are obtained from the florist fail to remain entirely true to type. The most familiar manifestation of this aberration occurs in some of the division forms: thus, in a plant of some twice or thrice pinnate variety there may appear one or more once pinnate leaves, and similar leaves may continue to be developed together with the typical leaves of the given variety. The resultant plant often shows a distressingly mixed or mongrel appearance. This sort of reversion is, however, only one of several types, the others being relatively frequent in florists' establishments. The purpose of the present paper is to describe these various types of reversion, both the products and, as far as possible, the processes. The term "reversion," which will be used interchangeably with "regressive variation," is here applied to any modified form which shows a change from some progressive variety back toward *bostoniensis*. Thus, the production by a variety with leaves twice or more pinnate of leaves less divided than the typical form is called a reversion. The production by a dwarf form of a plant with larger leaves is also considered as reversion, and likewise the reduction of ruffling. Broadly, we may recognize three manifestations of such reversion.

1. The simplest possible type is seen in the appearance of single aberrant, *i.e.*, reverting leaves among others which are typical. This is a common occurrence in varieties in the division series.

2. Again, we may find all the new leaves of a single crown or stem axis appearing reverted in form; *e.g.*, in a plant of a thrice-divided variety the typical leaves may be succeeded by once pinnate leaves developed one at a time until the former appearance of the crown is entirely altered.

3. The third type of reversion occurs when the change in character takes place in one of the branch reproductive shoots or stolons, and appears only when from this stolon new bud plants develop which manifest the new character (Pl. IX, fig. 1).

The first two types may be designated as "crown" reversion, partial and complete, and the second may be called "runner" reversion. Only the crown type may be actually watched in the external manifestations of the process. Runner reversion is known only in its products, *i.e.*, the new forms developed as bud plants on lateral stolons. The cytological changes are scarcely even a matter for conjecture, the evidence dealt with here being entirely macroscopic.

---

[3] It may be of interest to some to know that a considerable set of varieties of these Nephrolepis sports may be obtained from John Lewis Childs, Floral Park, New York. At present the Childs catalogue lists 30 different forms representing all types of variation listed in the chart, both progressive and regressive. The stock plants are grown separately, and the identification of the varieties offered may be generally relied upon.

The study of regressive variation offers one distinct advantage over that of progressive variation in the fact that the former is sufficiently common to be frequently observed, and may thus be studied experimentally. This suggests the possibility of determining an external cause for reversion. So far no definite experiments have been carried on, but there are some suggestive facts connected with the possible relations between cultural conditions and the occurrence of some of the reversions.

As with the first paper, the data for the present article have been obtained in two ways: first, by the further extension and study of the collection of Nephrolepis at the Brooklyn Botanic Garden. Some of the reversions here described have been developed at the Garden under observation. Second, visits to florists have been continued for the opportunity of seeing hundreds of thousands of plants. During 1916 these visits were made with the aid of a grant from the American Association for the Advancement of Science. A second similar grant was made in 1917 and has served in the preparation of this paper. Acknowledgment is also made to the Bureau of Plant Industry, specifically to Messrs. Peter Bisset, Wilson Popenoe, and David Fairchild, for assistance in the collection of wild forms of Nephrolepis from various parts of the tropics.

Special acknowledgment is again tendered the Brooklyn Botanic Garden for the very satisfactory facilities for this study which have been enjoyed.

Description of the Regressive Varieties

It will forward the description of the regressive variations to state here one of the conclusions based on the detailed observation of all the forms: namely, regressive variations may be expected to occur in any of the progressively developed varieties. This statement has not been checked for all the one hundred varieties known to exist, nor even for the forty listed in the genealogical chart attached herewith, but it has been found true for so many of them that it is a reasonable assumption that it may be characteristic of all.

This multiple origin of reversionary forms makes it impossible to arrange their descriptions in as simple a system as was used for the progressive varieties. With the latter it was merely a case of arranging them in a genealogical table, and describing for each the changes or advances in form it manifested. Each new kind in a series was produced by the intensification of some character of the parent type, or by the appearance of some other of a few well marked characters. Each reversion represents similarly some definite divergence from its parent form, but instead of one common ancestral type there are forty to one hundred possible ancestors. The reversion of any given variety is thus a distinct problem for study and description, and is related to other reversions only as it may produce analogous or comparable forms. Because of this extremely complex material, the present paper will deal mainly with what are believed to be representative types of regressive reversions.

GENEALOGICAL CHART OF PROGRESSIVE SPORTS OF BOSTONIENSIS

Black face type indicates division sports. Italics indicate sports showing ruffling. Plain Roman type indicates dwarfing. Small capitals indicate other types of variation.

- EXALTATA. *bostoniensis*
  - Piersoni....
    - **Barrowsi**..... **Whitmani**.....
      - WHITMANI “IMPROVED”
      - **Whitmani compacta**
      - Elmsfordi
      - **magnifica**
      - **gracillima**
      - **Goodi**
      - **? Smithi**..... **Craigi**
      - SUPERIOR
    - SPLENDIDA
    - superbissima..muscosa
    - **elegantissima**. ELEGANTISSIMA “IMPROVED”
    - **elegantissima compacta**.... **sport like Whitmani compacta**
    - PIERSONI “IMPROVED”
  - **Anna Foster** **?? Amerpohli**
  - *Harrisi*...... *Wm. K. Harris*
  - *Roosevelti* ..
    - *Sport like Wm. K. Harris*
    - Teddy Jr...
      - Dwarf sport .... FISH-TAIL SPORT
      - Randolphi
      - Smallest dwarf
  - Scotti.....
    - FALCATA
    - Wagneri
    - **Scholzeli**..... **Three-pinnate sport**
  - Giatrasi....
    - New Jersey
    - **Queen Sophie**
  - GRETNAI

Since the progressive varieties all originated from one form, *bostoniensis*, it will be interesting to consider first the primary sports of that fern. To what extent has the original process been reversed? After that, the reversion of the different grades of the division series will be considered. What are the products of the reversion of twice pinnate forms, of thrice pinnate forms, etc., etc.? Are the progressive steps each retraced in reversion? Can a four-times-pinnate variety produce thrice, twice, and once pinnate reversions, or only one of these? Similar questions suggest themselves in connection with the dwarfing and ruffling series, and will be considered in order.

Reversions of the Primary Sports of Bostoniensis

Before the reversions of the primary sports of *bostoniensis* are dealt with, it will be worth while to consider the possible reversion of *bostoniensis* itself. *Bostoniensis* differs from the wild species, *exaltata*, from which it must have originated, in certain vegetative characters—it has laxer, broader, more numerous leaves—and also in producing only abortive sporangia. The wild species is abundantly fertile, and the spores germinate readily. In *bostoniensis*, however, the sporangia abort at an early stage, apparently before the tetrad division. The resulting sporangia are small and shriveled, but there are usually enough of them to produce fruit dots of considerable size and the gross appearance of fertility.

Of the actual reversion of *bostoniensis* to a fertile condition there is no authentic record. There have been many attempts on the part of florists to produce plants by planting the soral material and there have been a few reports of success, but the reports have told nothing of the condition of the plants from which the spores (?) were obtained, *i.e.*, whether the original plant had first reverted in reproductive and vegetative characters as well, or whether the fertile spores were a chance production on a plant otherwise like ordinary *bostoniensis*.

There is nothing inherently improbable in either of the above suggested contingencies. An approximation to vegetative reversion may frequently be seen in plants which have been grown in untoward conditions of light, temperature, and food. In these plants the leaves assume a stiffer, more erect habit, decidedly suggestive of the appearance of the wild species but without its condition of spore fertility. Favorable conditions cause a return to the normal *bostoniensis* characters. It may be noted, however, that real reversion might occur frequently in florists' establishments without being observed, because the difference in appearance between the sterile and the fertile sori is not calculated to attract the attention of the average grower, and also because a reverted plant would probably be discarded as undesirable.

The primary progressive sports of *bostoniensis*, as listed in the revised chart, comprise four types: (1) Division sports, *Piersoni* and *Anna Foster;* (2) Dwarf sports, *Scotti* and *Giatrasi;* (3) Ruffled sports, *Harrisi* and *Roosevelti;* and (4) a "fish-tail" or forked-leaf form, *Gretnai*. The possible and actual reversions may be best considered under the same categories.

Piersoni. The history of *Piersoni* is illuminating. I am indebted for the following facts to J. C. Trevillian, who has been in charge of the fern houses at F. R. Pierson's Tarrytown establishment since and before the origin of *Piersoni*, the first of a long line of Pierson varieties.

The first break from the once pinnate form came as a small two-leaved runner in a stock bed of *bostoniensis*. Both the first two leaves were twice pinnate. This small plant was set apart and most carefully cherished. As proved by later developments, it was worth many times its weight in gold. Its third leaf must have been a disappointment, for it represented a return to the once pinnate form and may therefore be considered the original example of reversion. Thenceforward the original *Piersoni* plant and the thousands of progeny of this variety have always shown a mixture of leaf types: *viz.*, typical twice pinnate, once pinnate, and intermediates on which entire, lobed, and divided pinnae may co-exist. Even a single pinna may show all three grades of division (Pl. V, figs. 1–6).

Not only is there variation in leaf division on a single plant at any given time, but there seems also to be seasonal variation or possibly variation according to different cultural conditions. In April, 1916, a group of about fifty stock plants of *Piersoni* was observed in the John Lewis Childs Floral Park greenhouses which were almost completely in a once-pinnate condition. The plants were growing set out in rich soil in a well lighted bench. Most of them possessed only once pinnate leaves, but their identity was not possibly in doubt both because records had been kept of the planting and because of subsequent history. Three of these stock plants possessing only once pinnate leaves were selected for special observation with the thought that they might represent permanent reversion to a once pinnate condition. After being potted up at the Botanic Garden they were given good conditions of lighting, etc. The new leaves which followed were mainly of the twice pinnate type. Since then, all the plants of this origin kept at the Botanic Garden are predominantly twice pinnate, although cultural conditions have not been continuously at the optimum.

The experience with the Childs plants just described bears out a conclusion which Trevillian has reached after twenty years' experience with Nephrolepis varieties: *viz.*, that during the less favorable cultural conditions of winter, all the division varieties tend to produce more reverted leaves than in the more favorable six months from April onward. It is a well established fact that all *bostoniensis* varieties show a recognized slowing up in the number of new leaves produced during winter, and apparently associated with this is a tendency to produce leaves with less division. It may be concluded, then, that the maximum development of the special characteristics of any form depends upon an optimum of cultural conditions, and that it is therefore with Nephrolepis varieties partly a matter of season. The Childs plants above cited presented an extreme case of seasonal fluctuation.

The condition of *Piersoni* with respect to the amount of leaf division may be described as a state of "fixed instability." Taken as a whole, the variety represents a distinct and definite saltation from *bostoniensis*, but the jump or variation was not all the way to a stable twice pinnate type although the predominant leaf form throughout the year and normally at any time of the year is twice pinnate. The amount of reversion noted in the Childs plants referred to above is exceptional. Twice pinnate leaves are usually in the majority at all times of the year. That the divided-leaf condition is fixed is well illustrated by the experience with the Childs plants just related. Although externally those plants had lost all indications of the twice pinnate character, continued cultivation showed that it had been only temporarily obscured. It seems a safe conclusion that the actual basis of the original *Piersoni* variation from *bostoniensis* must have been a protoplasmic change which maintained itself even though temporarily not evident in the gross characters.

The facts detailed thus far—the appearance of once pinnate leaves in *Piersoni* stock—represent no real regressive variation or reversion but rather fluctuation within the limits of the *Piersoni* type. There are, however, three possible types of unmistakable reversion which should be considered.

The occurrence of a complete and discontinuous reversion to a once-pinnate form is highly probable. The fact that there is no record of such a reversion does not alter the probability. The florist would not be likely to detect its occurrence. *Bostoniensis* is almost invariably grown wherever any of its varieties are in cultivation, and the appearance of a once pinnate plant among *Piersoni* stock ordinarily would be passed over as a misplacement of a *bostoniensis* plant. As will be described below, this type of permanent reversion is well known in other varieties.

Partial reversion to a condition of greater instability is postulated on the basis of the behavior of other varieties considered below. This change would result in a strain in which the number of twice pinnate leaves would be fewer than in the best strains.

The most interesting reversion attributable to *Piersoni*, or, indeed, to any other *bostoniensis* variety, is a plant of the *Piersoni* type of division, but one the exact origin of which is unfortunately shrouded in doubt. I obtained it from the New York Botanical Garden where it was being grown under the name of a totally different variety and species, *Nephrolepis hirsutula tripinnatifida*. Its earlier history is unknown, but two explanations of its origin are possible. It may represent a progressive twice pinnate sport from wild fertile *exaltata* of which the New York Garden has plants from several collections in Porto Rico; or it may represent a reversion in fertility from some plant of *Piersoni* which has been grown there for the nineteen or twenty years since its introduction. The latter explanation seems the more probable. In any event, it is unique among *Nephrolepis exaltata* forms in its fertility, and a particular interest attaches to it in the possible type of its spore progeny of which several separate sowings are being grown and will be reported on later.[4]

Anna Foster (Pl. V, figs. 7–12) represents a twice-pinnate form also in a state of unstable division like that of *Piersoni* but even more pronounced. It was the first of all the Boston fern sports to be reported, but seems always to have been so unstable and so much less attractive than *Piersoni* that it never gained much general popularity. In well developed plants about half the leaves show the double pinnation, but this is never so completely developed as in good *Piersoni* leaves. The best leaves are partly once pinnate, and most of the divided pinnae are incompletely divided. At the present day, twenty years after its appearance, there has come down one strain attributable to *Anna Foster* which shows only an occasional twice pinnate leaf. Such a plant presumably represents reversion from the more completely divided original type.

---

[4] For purposes of reference and discussion, I have assigned the name "*fertilis*" to this form. Technically its name should read, *Nephrolepis exaltata bostoniensis* var. **fertilis**.

Harrisi and *Roosevelti*. These two varieties, possessing almost identical leaf forms, may be dealt with together as far as possible reversions are concerned. No reversions are known. The conditions in this respect are almost exactly like those discussed in connection with the possible complete reversion of *Piersoni* to a once-pinnate form. Florists who grow the ruffled forms almost always grow *bostoniensis* as well, since the cultural conditions required are the same. If a complete reversion should occur, it would pass unnoticed or be interpreted as a chance *bostoniensis* plant accidentally associated.

Scotti. Exactly the same conditions prevail with *Scotti* as with *Roosevelti* and *Harrisi*. No reversions have been detected, not even occasional fluctuating leaves.

Gretnai. The fish-tail form, *Gretnai*, shows fluctuations in the degree in which its pinnae and leaf tip fork, but the character is always present to some extent. No complete reversion to the normal Boston-fern type has been noted.

Giatrasi. This fern represents one of the most distinct of all mutations from *bostoniensis* shown in the primary sports. *Piersoni*, while differently divided, has the same habit and size, and produces continually some similar leaves. *Giatrasi*, on the contrary, differs so markedly in size, habit of growth, and leaf characters that its separation as a "species" could easily be justified. Its leaves attain a length of no more than one third that of *bostoniensis;* the petioles are darker colored and wiry, those of *bostoniensis* being greenish and herbaceous; the pinnae are shorter in proportion, blunter, and wavier, the midribs are often sinuous, and the plant is notably slower in growth.

Giatrasi (Pl. VIII, fig. 2) does not produce any single fluctuating leaves, but it has produced as a runner sport (at the Giatras establishment) a form which may well be considered a reversion. This new form was introduced to the trade under the name of the "*New York*" fern (Pl. VIII, fig. 1). In characters it is intermediate between the Boston fern and *Giatrasi*, possessing in considerable degree all the characters of the latter fern except that it is considerably larger although never equalling *bostoniensis* (Pl. VIII, fig. 3) in height. There is no indication of "fixed instability" here. Both *Giatrasi* and the "*New York*" fern are discontinuous and stable sports, without fluctuations *inter se.*

The case of the *New York* Nephrolepis emphasizes a fact true in general for reversions: complete return to all the characters of the parent form rarely if ever occurs. The original progressive variations were not mere fluctuating changes about a mean, but decided and permanent changes which must find their explanation in some cytological alteration of definite character.

Summarizing, we may note the following types of reversion among the primary progressive sports of *bostoniensis:*

1. A reduction in the stability of the original progressive change, as shown by a permanent decrease in the proportion of divided leaves in *Anna Foster* and probably also in *Piersoni.* It is possible that this type of reversion should be considered as a fluctuation.

2. A possible reversion (mutation) to a fertile condition, with the *Piersoni*-like form from the New York Botanical Garden as an illustration (Pl. V, figs. 13–17; Pl. VI, fig. 6).

3. A reversion (mutation) from a dwarf form to a size intermediate between the dwarf and *bostoniensis* (Pl. VIII).

N.B. The production of single atypic leaves does not constitute reversion but rather merely part of the normal variability of a given plant (Pl. V, figs. 1–17).

Reversions of the Secondary Sports of Bostoniensis

Reversions of superbissima (Pl. VIII, fig. 6).

The simplest examples of reversion among the secondary sports of *bostoniensis* have occurred in plants of the variety *superbissima.* This is a dwarfed sport from *Piersoni,* and differs from the latter form almost entirely in a foreshortening of the rachis and of the midribs of the pinnae. The actual amount of green tissue does not seem to be proportionately reduced. As a result of this brachytic type of dwarfing, the leaves have a crowded and congested appearance. In type of division and stability of leaf division, *superbissima* seems to be exactly like *Piersoni.* In the course of a year any given plant is sure to produce a good many once-divided leaves. Two distinct reversions have been noted and are represented by forms which have been continuously cultivated.

The first of these was introduced as a new variety by Pierson under the name of *viridissima* (Pl. VIII, fig. 5), given on account of its dark green color, presumably the result of the congestion of leaf tissue. This form is of the same size as *superbissima* and has the same rigid thick rachides, but is entirely once pinnate like *bostoniensis.* In other words, it represents a reversion in one of the two progressive characters of *superbissima,* that of leaf division. The plants of *viridissima,* as grown for some years, have continued constant, and show no indication of fluctuating variations toward a more divided form.[5] A second instance of this reversion has also taken place at the Brooklyn Botanic Garden in material of *superbissima* obtained originally from the city greenhouses of Fairmount Park, Philadelphia. So far it has not been sufficiently grown to allow an opinion as to its identity with Pierson's *viridissima.*

From *viridissima*, although no fluctuating variation has been observed, there has developed in at least two establishments a further reversion of stable character, in this case one of size. This new form (Pl. VIII, fig. 4), which has not received any name, is taller and laxer, thus intermediate in size and habit between *viridissima* and normal *bostoniensis.* It has not, however, made complete return to *bostoniensis* size but is comparable to the "*New York*" fern, described on a preceding page as a reversion from *Giatrasi.* Like that form, it is stable in its characters, and it may here be noted as a general observation that the mutations showing reduced size are invariably more stable than those presenting differences in amount of leaf division. This semi-dwarf mutation from *viridissima* was first noted by Trevillian in the Pierson establishment. Since then I have found it also in the greenhouses of Peter Wagner of Brooklyn.

From *superbissima* there has developed directly another reversion in size (Pl. VIII, fig. 7), but which has retained the double division of both *superbissima* and *Piersoni.* This also was first noted in the Pierson greenhouses where it has developed in *superbissima* stock more than once. It has also occurred in the greenhouses of John Lewis Childs at Floral Park, and likewise at the Brooklyn Botanic Garden. A description of the circumstances of this last occurrence is worth recording.

In a pot of *superbissima* which included three or four crowns, there developed in one crown two leaves considerably taller and looser in division. The crowns were then potted separately for observation. That containing the two taller leaves developed more of the same sort of leaves, becoming eventually intermediate in size between *superbissima* and *Piersoni.* Additional plants were raised from it of the same sort, and the form remained stable in further cultivation.

It should be noted here that the several reversions of this particular type have not resulted in exactly identical forms. The different plants are all intermediate between *superbissima* and *Piersoni* but there are some variations in height and shape of the segments.

Special interest attaches to the three reversionary forms above described because of their very definite character. Each represents a single return toward the original Boston-fern type. In all three cases the new forms were immediately stable in their own type. That is, there was no tendency to fluctuate toward the parent forms. It is also of interest, as has been noted by Babcock and Clausen (*Genetics in Relation to Agriculture*, pp. 315, 316), that these changes in leaf form and size occur independently of each other, indicating that the protoplasmic basis of the changes is also distinct—"to factor mutations in vegetative reproduction."

---

[5] Since the above sentence relating to *viridissima* was written, a further variation has taken place in this form, first noted in the summer of 1921. In the only plant of *viridissima* being maintained, what appears to be a definite return to the characteristics of *superbissima* has occurred, so that there is now no authentic plant of the original Pierson *viridissima* sport in the Botanic Garden collection.

Reversions from elegantissima-compacta.

The form *elegantissima-compacta* has given rise to more distinct reversions than any other variety. It may be recalled here that it, like *superbissima*, represents a doubly progressive sport from *Piersoni*, showing both increased leaf division and reduction in size. In its dwarf character it does not have the foreshortened and congested aspect of *superbissima*, and its leaves are somewhat more divided, being twice pinnate-pinnatifid (Pl. IX, fig. 2; Pl. X, figs. 1–3). This division is also considerably more stable than that of *Piersoni* or *superbissima*, although reverting leaves occur not infrequently, and consequently seasonal changes may also occur. These, however, do not affect the type of the plant, which continues year after year to hold the characters of the original variation. By analogy from *superbissima*, we should expect at least the two types of reversion found in that plant, the production of a taller twice-pinnate plant and of a once-pinnate form. As a matter of fact, both these expected forms and one other have developed.

The simplest form is a complete reversion to the once-pinnate condition, first found at the Pierson establishment and sold by them under the name "Dwarf Boston" (Pl. X, fig. 4). This form is of about the same size as the primary progressive sport *Scotti*, and under some conditions rather closely resembles this. It has typically, however, more the appearance of *bostoniensis* in leaf form and habit. It is stable in that no divided leaves are produced from it. As it is somewhat taller than *elegantissima-compacta*, it is to be considered also a reversion in size as well as in division.

Another distinct and definite reversion has been introduced under the name of "*John Wanamaker*" (or *Wanamakeri*) by Robert Craig of Philadelphia (Pl. X, figs.13–18). This is an incomplete reversion in division in which some of the leaves are entirely once pinnate but considerably ruffled and wavy, while others are more or less lobed or even twice pinnate but also showing the ruffling. The leaves are taller and narrower than those of *elegantissima-compacta*, even when divided. The variety seems to represent reversion toward double division reaching only an intermediate condition. In its ruffled character it might be considered to present a progressive sport in this particular, but it seems more reasonable in this case to interpret this ruffling as a modification of leaf division.

This same form has arisen several different times at the Pierson greenhouses and also at Giatras's place in West Hoboken. These different examples of this mutation vary a little in form and size, but are, in general, very like each other.

A plant of this type obtained originally at Pierson's and set out in an open stock bed at the Brooklyn Botanic Garden underwent an interesting change in that it returned entirely, in crown and runners, to apparently the original type of *elegantissima-compacta*. There might be reason for suggesting that the growth in an open bench had something to do with it except for the fact that in many thousands of plants grown under the same conditions at Craig's I did not see any similar break. The explanation is probably found in the possibility that the particular number which I obtained from Pierson did not really represent a stable sport or mutation but rather a fluctuation of a type which will be described below.

From the *Wanamaker* type there has developed at Pierson's a reversion to a completely once pinnate unruffled form, corresponding to "Dwarf Boston" in general appearance and size (Pl. X, fig. 5), but not sufficiently cultivated to warrant any general opinion as to its characters. It corresponds to the reversion from *viridissima* described above since it represents a second step back toward *bostoniensis*.

In addition to the above mentioned, Trevillian has also detected among the plants in his charge another *elegantissima-compacta* mutation which approaches *Piersoni* in characters, *i.e.*, it is twice pinnate and tall. Two examples from Pierson's have been grown at the Botanic Garden (Pl. VI, figs. 2, 3). By reference to the illustrations it will be seen that there is some little difference in the shape and size of the ultimate segments of these two types. Both, however, are generally like the *Piersoni*-like reversions produced from *superbissima*, and, it may be added, like other similar reversions from progressive sports of a higher order of leaf divisions. A series of leaves of six of these types, produced by mutation from five different progressive sports representing several different grades of leaf division, is illustrated in Plates VI and VII. Thus figure 1, Plate VI, shows a reversion from *Smithi*, a four-pinnate form. Attention is called to the fact that the six types, although alike in division, vary noticeably in outline and carriage of the pinnae, in texture, and in minute characters.

When it is recognized that each of the twenty derivative mutations from *Piersoni* noted on the chart may give rise directly or indirectly to reversions of a *Piersoni* type, the possibilities of a confused tangle of forms, practically impossible to differentiate by description, will be realized. What explanation could the ordinary systematic examination of such a group of forms bring forth? Some systematists would explain the variation as a set of closely related intergrading forms, connecting the extremes as parts of one single "species." With fertile sexually reproducing forms, the suspicion of hybridism would certainly attach. Especially would this apply in such cases in which the reversion was intermediate in form and other characters. Obviously, if we may consider these vegetative mutations as analogous to variations among wild forms which appear similar, we may see adequate reason for caution in making generalizations regarding complexes of wild forms.

The reversions of *elegantissima-compacta* described above all belong to the category of stable forms, presenting distinct and definite changes in leaf form and division. The kind of reversion mentioned earlier in the paper as reduced stability of division ("fixed instability") has also occurred, and one case has been under continuous observation and cultivation for several years.

A plant of typical *elegantissima-compacta*, obtained directly from its place of introduction, the Pierson establishment, was one of the original group of *bostoniensis* varieties to be received in 1915, and in fact was designated "no. 1" in my accession series. With the other forms then at hand, it was planted out in open soil in a bench for reproduction by runners. Among its numerous progeny in that first cultivation was one small plant with leaves almost entirely once pinnate. In the spring of 1916 (March) this was planted in a large square pan for further propagation with the idea that it might prove to be a stable once pinnate reversion. At this time it consisted of six leaves (Pl. IX, fig. 1, shows a similar plant), entirely once pinnate with the exception of one which was shallowly lobed on some pinnae. By August the pan had become full of small plants which were separately potted and grown for eleven months. Although the stock crown had been almost completely once pinnate, these runner plants were in general like typical *elegantissima-compacta* in leaf division, with scattered once pinnate leaves mixed in. In July, 1917, the stock consisted of fourteen plants in four- and six-inch pots, but not in the best condition, owing to crowding. Three sorts of plants could be distinguished among them. One plant with fifteen or more leaves was almost entirely once pinnate, only one or two leaves showing any double division. There is little doubt that this represented the original once pinnate crown first detected, but this cannot be absolutely assured. Nine plants were mainly twice pinnate as in typical *elegantissima-compacta*, but each had one or more once pinnate leaves. Four plants had leaves practically without any reversion.

The cultural conditions had been the same for all, so that the modifications observed must have taken place as a result of internal changes. Examples of the three sorts were kept for further observation, but their subsequent behavior was the same. The plant with mainly once pinnate leaves continued once pinnate in its original crown, but the secondary crowns produced in the same pot by runners to the number of eight had leaves mainly of the twice-divided type of *elegantissima-compacta*. In other words, the original once pinnate crown, produced as a runner variation, retained its leaf characteristics as its own new leaves developed, but gave rise only to new plants with more divided leaves, either like typical *elegantissima-compacta* or, in some cases, with the less stable amount of division, but with no real modification of leaf form (Pl. X, figs. 6–12).

This behavior is worthy of emphasis because it has been found to be of frequent occurrence in the study of reversion among other division forms.

On more than one occasion and with diverse varieties, attempts to reproduce some reverting leaf type by setting out the new form in bench for vegetative reproduction have resulted in the continuance in the new crown of its new characteristics but with the reappearance in the runners derived from it either of the original form of division or of that form with some reduced stability. The larger size of the reverted crown is typical generally of reversions, both temporary and permanent sorts.

With these facts in mind, exception must be taken to the conclusion adopted by Boshnakian (Jour. Hered. 7: 233. 1916) and repeated by Babcock and Clausen (*Genetics in Relation to Agriculture*, fig. 130 and explanatory description), interpreting a series of four connected Nephrolepis runners which show differences in form as four different mutations. The series shows respectively (1) a four-pinnate variety, *magnifica;* (2) a derived once pinnate plant; (3) a third generation (?) plant with unstable division, and (4) another third generation (?) plant like *magnifica*. Similar series of runners have occurred in my cultures (Pl. IX) which on further cultivation have resolved themselves into the three types of plants cited above, *viz.*, (*a*) second generation reverted crowns, usually only one; (*b*) third generation crowns, like the original variety in division and in stability of division; (*c*) other third generation crowns like the original variety but with more reverted leaves per plant. Unless the plants figured by Boshnakian were afterwards planted and found to continue distinct, it is not safe to cite them as so many mutations; rather, they seem to belong in the category of fluctuations. It has been my experience that while a plant of a divided variety may often produce occasional reverted leaves and sometimes a new runner plant with only reverted leaves, the production of a stable self-reproducing reversion, in other words a mutation, is most uncommon.

In general, any given division variety may be expected to produce in cultivation one of three types of plants. The most numerous—an overwhelming majority—will be of the form typical for the parent stock, the only modification being in occasional single reverting leaves such as occur in the stock plants themselves. Second, an occasional plant will be developed which will appear almost entirely reverted but which, on further reproduction, will develop new plants like those of the original form, or at most with some reduction in stability of division. Such plants would be classed as fluctuations. It is obviously important for growers in selecting crowns for propagation to avoid such undesirable stock plants. Very rarely, a third type of plant will appear, distinct not only in its own characteristics but also in its runner progeny, a real mutation. These are somewhat more common in the regressive direction than in the progressive.

There is a very interesting morphological problem involved in this matter of the production of new forms, whether of regressive or of progressive type. The problem can only be indicated here, but the main facts are worthy of note. It is a well known fact that the stolons of Nephrolepis originate in association with the leaves, one stolon being paired with each leaf in branching from the crown-stem axis. It should not be difficult to determine whether there is any association between leaf variations on the original crown and runner variation on the paired stolon. For example, do the stolons paired with reverting leaves tend to produce new plants showing a reduction in stability of leaf division in a manner analogous to the behavior in variegated Pelargonium?

The problem is not as simple as might at first appear from the above statement, because the relation of stolon to old and new plants is not entirely simple. The stolon from an original stock plant may bear along its several feet of length a considerable number of plants arising as lateral buds, generally on short spur branches. When the new bud plants are taken up for potting, each one may retain some portion of the parental stolon capable of continued bud reproduction. The progeny of such a plant would then consist of new plants from the original parental stolon together with others from the new stolons of the bud plant, *i.e.*, "sisters" and "daughters." With the possibility of such complications, the doubts expressed with reference to the relationships of the series of four connected plants figured by Boshnakian will be readily appreciated.

A not infrequent anomaly in the behavior of the stolons is what appears as a dichotomy of a stolon in which one half becomes a leaf without any evident associated stem axis while the other half continues as a normal stolon. I have seen three successive leaves produced in this manner on one stolon. Sometimes the stolon growing point is lost with the formation of a single leaf, and the appearance is given of the transformation of a stolon into a leaf. I have made no determination of the stelar behavior in these cases.

Reversions of other secondary sports.

Only one other secondary sport of *bostoniensis* has been recorded as having given rise to a reversion of the mutation type, another Pierson variety, *elegantissima*. This is a variety much like *elegantissima-compacta* in leaf division, but lacking the dwarf character of that form. Robert Craig of Philadelphia has reported and introduced as a new variety, named *robusta*, a form which has almost complete agreement in characteristics with plain *Piersoni* (Pl. VI, fig. 5). If its origin is correctly given, it is to be grouped with the other *Piersoni*-like reversions which have already been discussed.

Summarizing the facts recorded for the reversions of the secondary sports, we find the reversionary behavior similar to that described for the primary forms.

1. Reduction in the stability of the original progressive change as shown in the degree of division and in the proportion of divided leaves was experimentally demonstrated. Furthermore, this reduction was shown in *elegantissima-compacta* to pass through an almost completely reverted

form, and then to return in the next generation either to the original typical form or to one with reduced stability of division. The point is, there are cases of apparent reversion in which the new (*i.e.*, reverted) form seems incapable of reproducing its new type. Its runner progeny do not "breed true" but return in different degrees to the form from which the reversion originally sprang. The possibility suggests itself that such unstable variations may correspond in kind to the variations noted as probably due to seasonal changes. It may be that as reverted leaves appear to be more frequent during winter, long continued observation might also show that the temporarily reverted runners, the sort incapable of reproducing their kind, may also have a seasonal frequency.

2. Discontinuous variation in a regressive direction affecting the leaf division has been found in a number of forms: in *elegantissima*, to *robusta*, a *Piersoni*-like form; in *elegantissima-compacta*, in two steps through *Wanamaker* and to a once-pinnate form; also in *elegantissima-compacta*, in one step, to "Dwarf Boston," once pinnate; in *superbissima*, to *viridissima*.

3. Discontinuous variation, reversion in size: in *elegantissima-compacta*, to *Wanamaker*, and to a *Piersoni* type; in *viridissima*, to a taller form, intermediate in size between *viridissima* and *bostoniensis*.

4. No evidences of reversion to a fertile condition have been noted.

The characteristics and behavior of reversions from tertiary and higher degree progressive sports of *bostoniensis* will be left to a future paper for description.

Brooklyn Botanic Garden

EXPLANATION OF PLATES

Plate V

Fluctuating reversion: pinnae showing range of fluctuation in three forms.

Figs. 1–5. Pinnae from one leaf of *Piersoni*, ranging from typical twice pinnate division (fig. 1) through intermediate forms (figs. 2–4) to once pinnate type (fig. 5)

Fig. 6. Simple pinna from another leaf of the same plant of *Piersoni* as shown in figures 1–5.

Figs. 7–10. Pinnae from one leaf of "*Anna Foster*."

Figs. 11, 12. Pinnae of *Anna Foster*, taken from another plant.

Figs. 13–17. Pinnae of fertile *Piersoni*-like reversion, var. *fertilis*, showing same sort of fluctuation as in true *Piersoni*.

Plate VI

Regressive mutations: leaves of twice pinnate reversions from different progressive forms.

Fig. 1. From *Smithi*, a four-times-pinnate form.

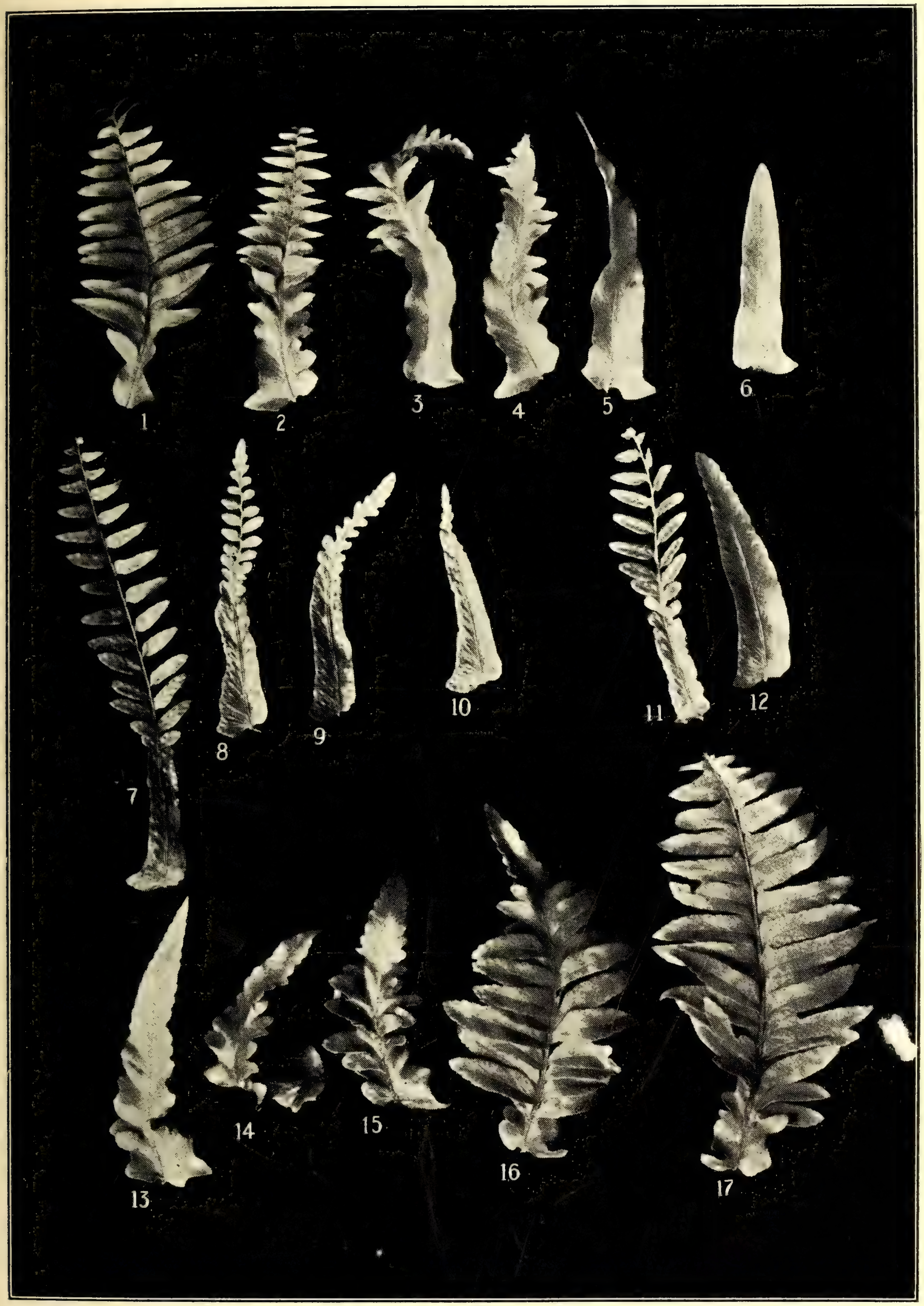
Fig. 2. From *elegantissima-compacta*, itself twice pinnate, pinnatifid.

Fig. 3. Also from *elegantissima-compacta*, but a distinct form.

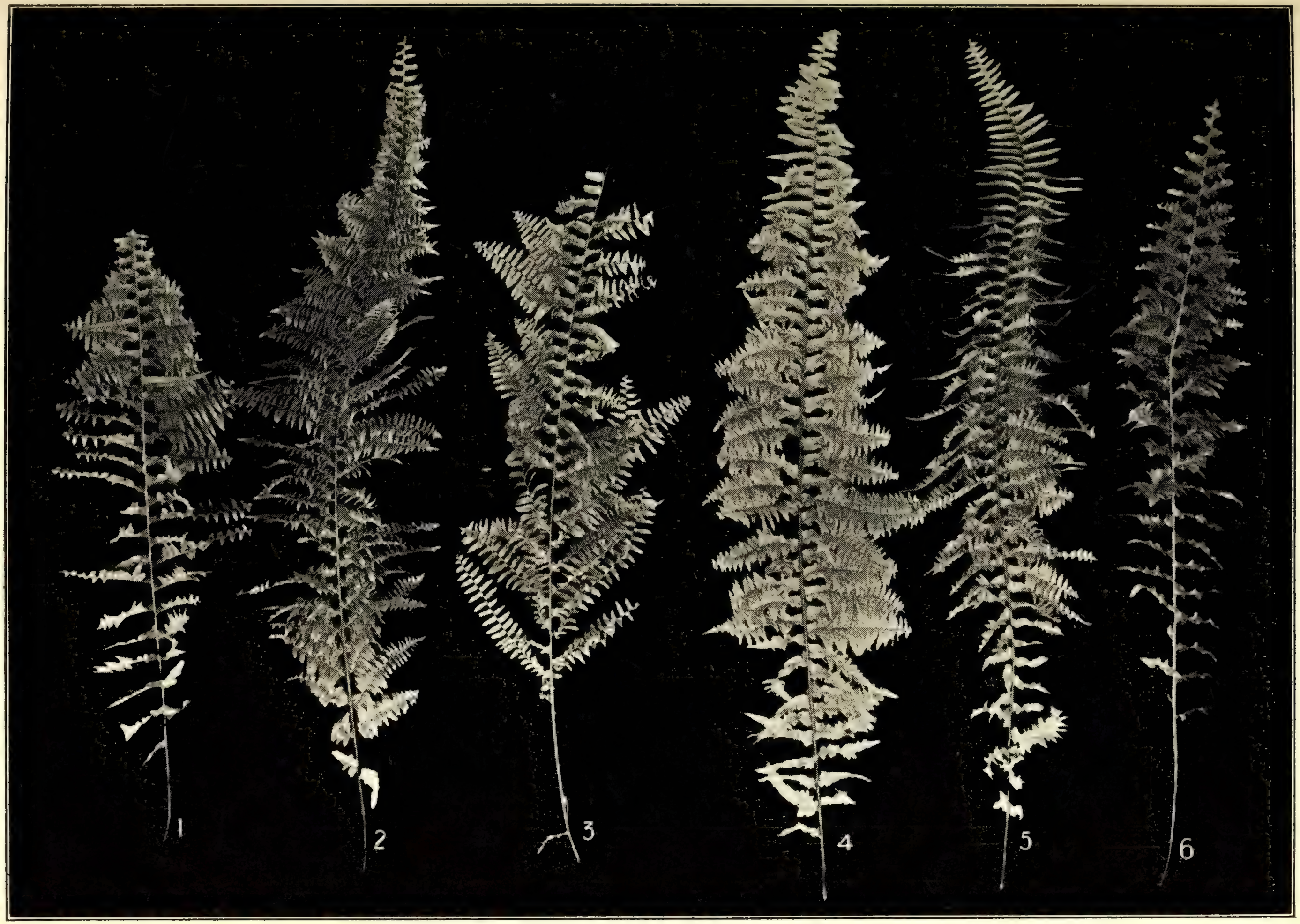
Fig. 4. From *superbissima*, a reversion in size (see also Plate VIII).

Fig. 5. *Robusta*, introduced by Robert Craig, Philadelphia; a reversion reported from *elegantissima*.

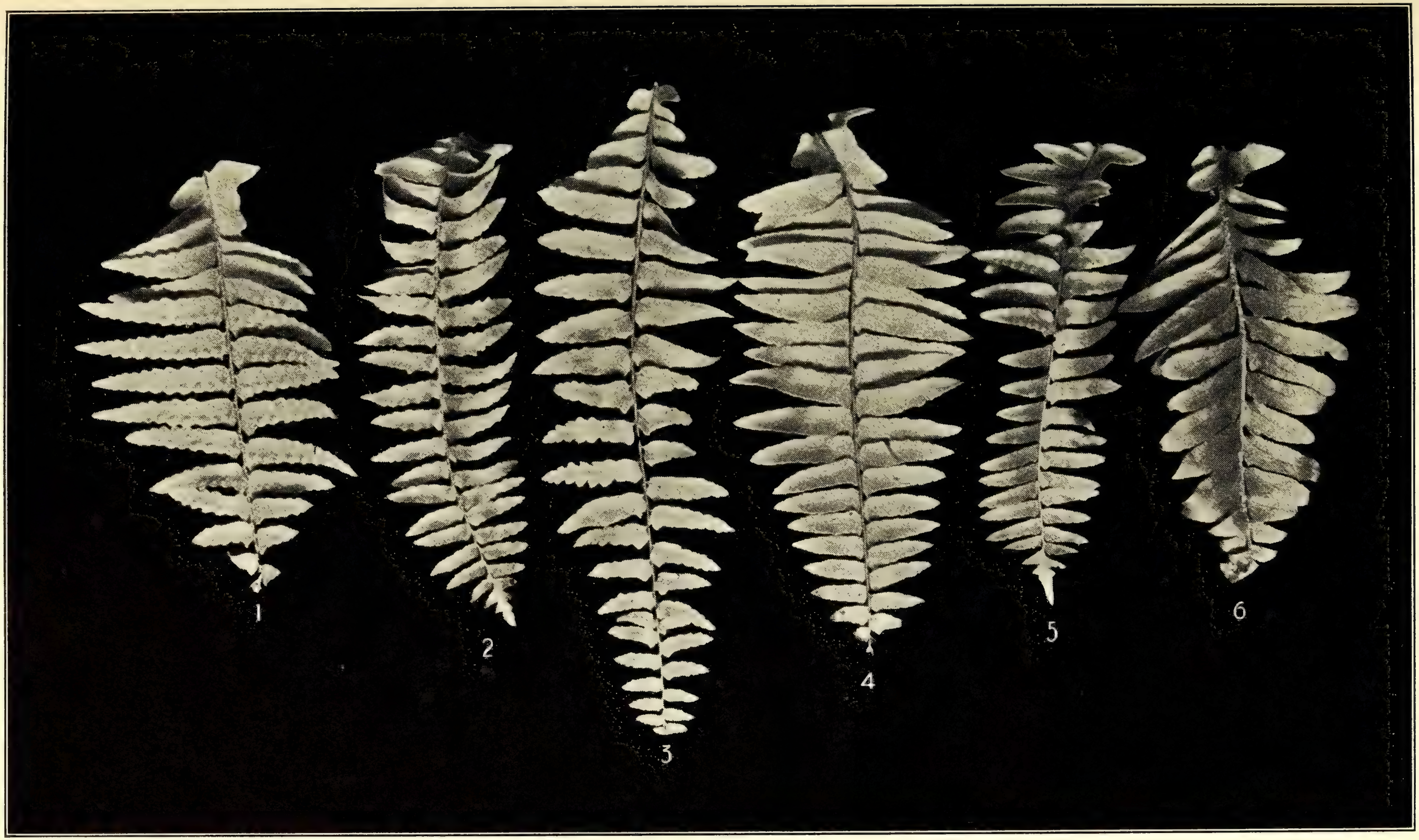
Fig. 6. Var. *fertilis*, possibly reversion from *Piersoni*.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

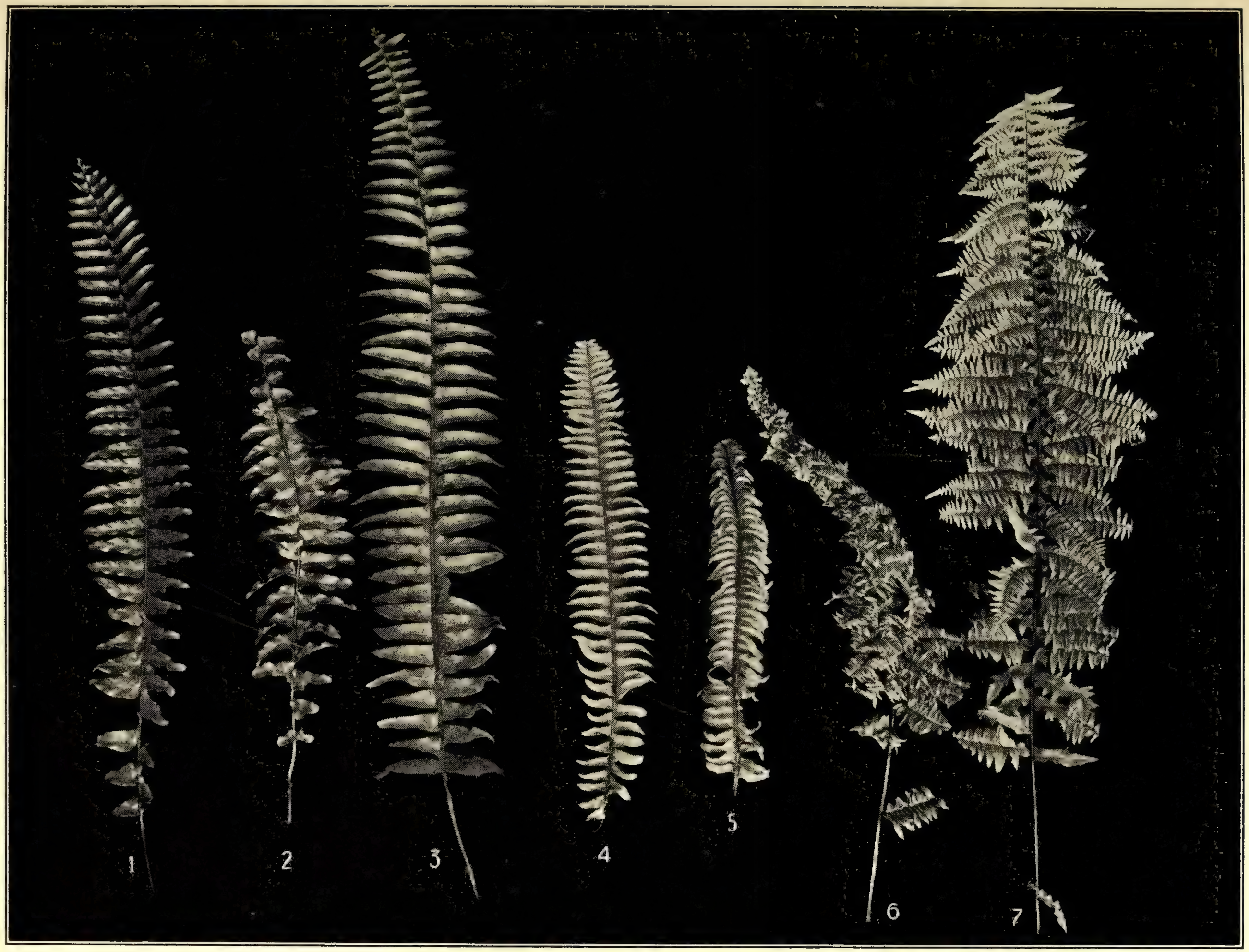
BENEDICT: VARIETIES OF NEPHROLEPIS



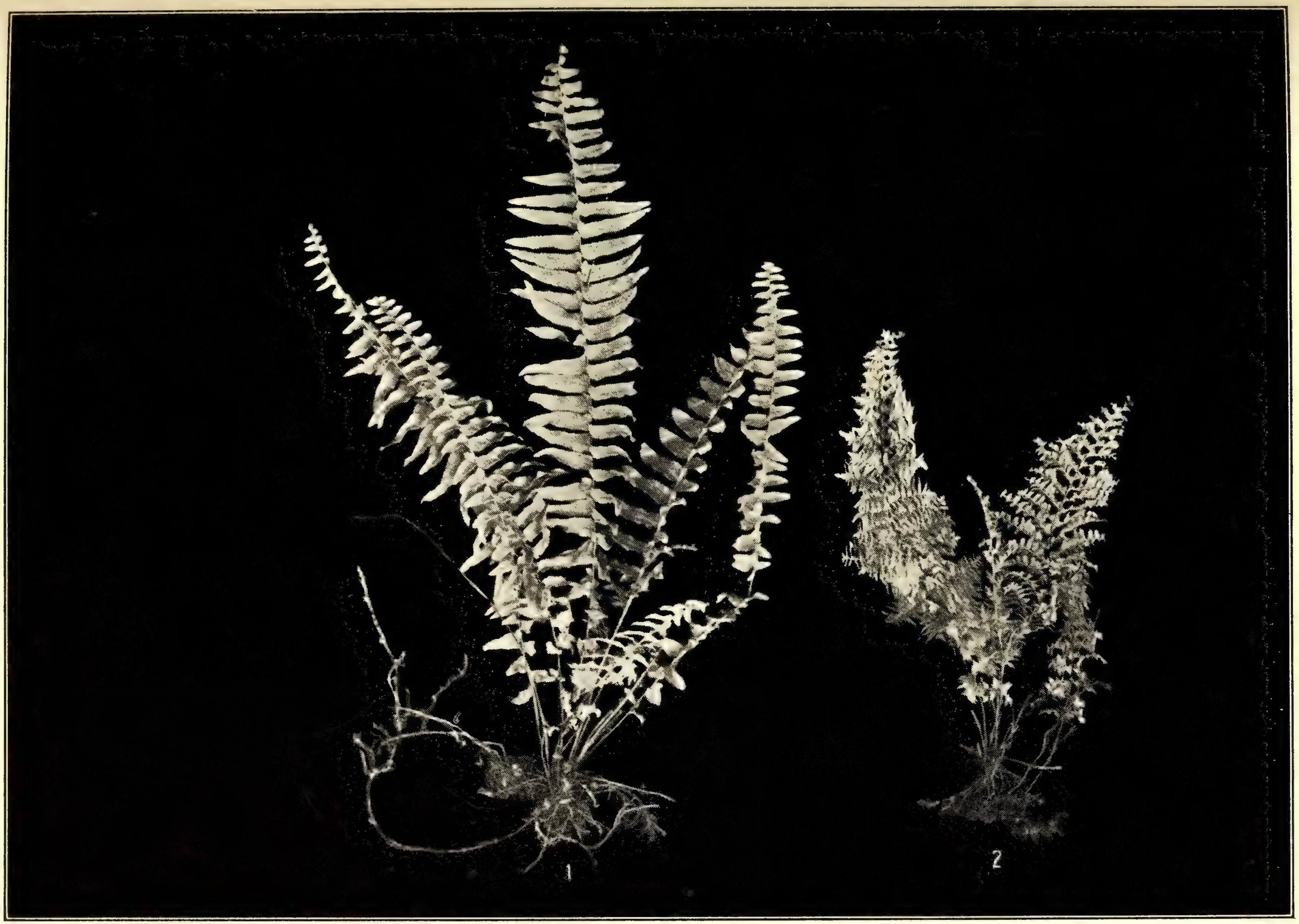
BENEDICT: VARIETIES OF NEPHROLEPIS



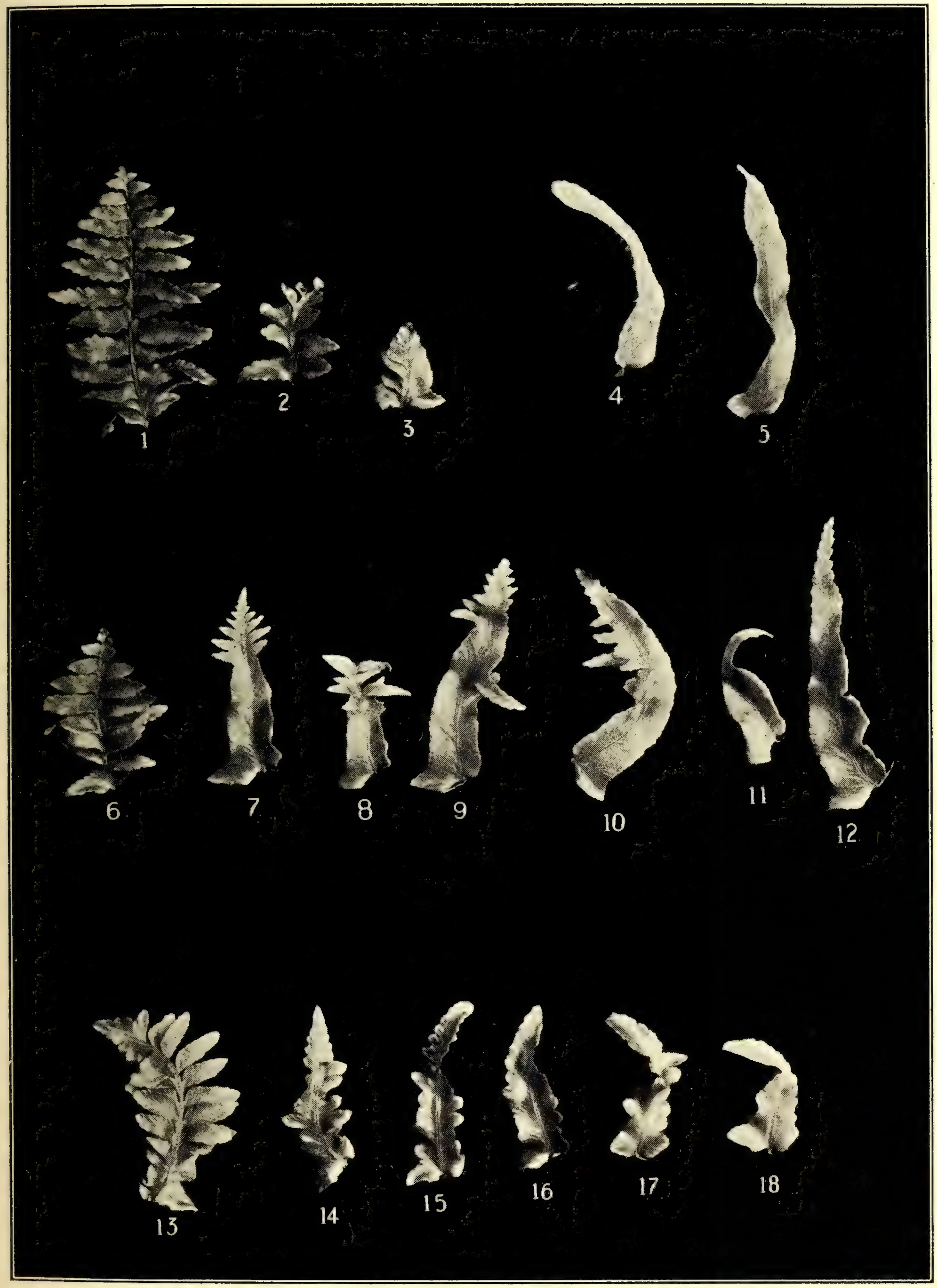
BENEDICT: VARIETIES OF NEPHROLEPIS



BENEDICT: VARIETIES OF NEPHROLEPIS



BENEDICT: VARIETIES OF NEPHROLEPIS


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

Benedict: Varieties of Nephrolepis

PLATE VII

Regressive mutations: pinnae of leaves shown in Plate VI.

FIGS. 1–6. Represent respectively the same forms as shown in Plate VI, figures 1–6.

PLATE VIII

Regressive mutations: leaves of forms showing reversion in size and in division.

FIG. 1. "*New York*" fern, a regressive mutation from *Giatrasi* (fig. 2), introduced by George Giatras, West Hoboken, N. J.

FIG. 2. *Giatrasi*, dwarf primary sport from *bostoniensis*.

FIG. 3. *Bostoniensis*, for comparison to show intermediate size of *New York* fern.

FIG. 4. Unnamed reversion, from dwarf once pinnate *viridissima* (fig. 5).

FIG. 5. *Viridissima*, introduced by F. R. Pierson, Tarrytown, derived by reversion in division from twice pinnate *superbissima* (fig. 6).

FIG. 6. *Superbissima*.

FIG. 7. Unnamed reversion in size, but not in division, from *superbissima*.

PLATE IX

Fluctuating reversion: bud plants showing unstable regressive variation.

FIGS. 1, 2. Sister plants of an unstable strain of *elegantissima-compacta*. FIG. 1, entirely once pinnate, except for a few pinnae on one or two leaves which show double division. FIG. 2, plant showing typical form of *elegantissima-compacta*.

PLATE X

Fluctuation and mutation of *elegantissima-compacta*.

FIGS. 1–3. Pinnae from one leaf of *elegantissima-compacta*.

FIG. 4. Pinna of "*Dwarf Boston*," introduced by F. R. Pierson as a stable reversion from *elegantissima-compacta*.

FIG. 5. Unnamed once pinnate mutation from *Wanamaker* (see figs. 13–18).

FIGS. 6–12. Pinnae selected to show range of fluctuation in an unstable strain of *elegantissima-compacta* (see figs. 1, 2, Pl. IX).

FIGS. 13–18. Pinnae selected to show range of fluctuation in *Wanamaker*, a regressive mutation of *elegantissima-compacta* introduced by Robert Craig, Philadelphia.

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1922

President: HENRY C. COWLES
University of Chicago

Vice-President: MARGARET C. FERGUSON
Wellesley College

Secretary: I. F. LEWIS
University of Virginia, University, Va.

Treasurer: IRVING W. BAILEY
Bussey Institution, Harvard University

Councilors:

N. L. BRITTON
New York Botanical Garden

J. C. ARTHUR
Purdue University

C. E. ALLEN
University of Wisconsin

Any one actively interested in botanical work is eligible for membership Candidates for membership must be recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including one subscription to the AMERICAN JOURNAL OF BOTANY. Candidates proposed for membership, and approved by the Council, may receive one copy of the JOURNAL at $4.00 per year pending their election at the winter meeting of the Society.

The Little Things That Count Big In Owning Your Own Greenhouse

Ever gone into a hat store, knowing exactly the kind and shaped hat you want; and then have some smart Alec of a salesman try to sell you the kind HE likes?

Makes a fellow madder than a wet hen, doesn't it?

Suppose, however, that same salesman had told you something about some of his hats that meant better service to you—more satisfaction for your money—you then sort of feel towards that salesman as you would an old friend.

Salesman number two is our sort when it comes to selling a greenhouse.

We do sell greenhouses, but we first sell satisfaction.

Catalog for the asking.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

PHILADELPHIA
133 So. 12th St.

Vol. IX. APRIL, 1922 No. 4

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

CONTENTS

Hydrogen-ion concentration in its relation to wheat scab . . . . E. F. Hopkins 159

On the physiological balance in nutrient solutions for plant cultures. W. F. Gericke 180

Vegetative vigor of the host as a factor influencing susceptibility and resistance to certain rust diseases of the higher plantsI . M. A. Raines 183

Rots of early strawberries in Florida and southern California. Neil E. Stevens 204

Smithsonian Institution
MAY 17 1922
National Museum

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
At 41 North Queen Street, Lancaster, Pa.
LONDON AGENTS
WHELDON AND WESLEY, Ltd.
28 Essex Street, Strand, London, W. C. 2

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN
JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
*The Thompson Institute
For Plant Research, Inc.*

H. H. BARTLETT,
University of Michigan

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ARTHUR H. GRAVES,
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

I. F. LEWIS,
University of Virginia

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Postage to Canada, 20 cents; to other foreign countries (except Mexico and Cuba) 40 cents a volume on annual subscriptions. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume, post free.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. 9 APRIL, 1922 NO. 4

HYDROGEN-ION CONCENTRATION IN ITS RELATION TO WHEAT SCAB[1]

E. F. HOPKINS

(Received for publication July 9, 1921)

That the reaction of the soil affects the severity of certain soil-borne plant diseases such as potato scab and the club-root of cabbage has been known for many years. That the hydrogen-ion concentration of the soil solution, however, is the controlling factor was not appreciated until Gillespie (1, 2) demonstrated that the soil solution has a definite hydrogen-ion concentration which can be easily determined, and Gillespie and Hurst (3, 4) showed that the hydrogen-ion concentration of the soil solution is apparently the controlling factor in the incidence of potato scab. The demonstration of the close relation between hydrogen-ion concentration and this soil-borne disease suggests that a complete and thorough investigation should be made of the relation of hydrogen-ion concentration to the development of other pathogenic organisms carried in the soil and to their ability to infect the host. In the present paper, a study is presented of the relation of hydrogen-ion concentration to the growth of *Gibberella Saubinetii*, the causal organism of wheat scab, and to the ability of this organism to produce seedling infection in wheat.

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF GIBBERELLA SAUBINETII (MONT.) SACC.

Comparatively little work has been done on the relation of hydrogen-ion concentration to the growth of pathogenic or non-pathogenic fungi. Clark and Lubs (5) grew *Aspergillus niger* on a mineral nutrient solution plus sucrose and found that the hydrogen-ion concentration on the seventh day was 2×10^{-2} ($p_H = 1.70$). Duggar, Severy, and Schmitz (6), in studying certain fungi in plant decoctions, found that in all solutions they used except sugar-beet and cornmeal decoctions, *Aspergillus niger* caused a shift towards the acid side equivalent to a hydrogen-ion concentration of about 10^{-3} ($p_H = 3.0$), while *Macrosporium commune* and *Glomerella Gossypii* generally evidenced a pronounced change in the other direction. With these latter two organisms the reactions of turnip, beet, and potato decoctions were changed from 10^{-6} to 10^{-8} ($p_H = 6.0$ to 8.0). The authors state that

---

[1] Published by permission of the Director of the Missouri Agricultural Experiment Station.

[The **Journal** for March (9: 93-157) was issued April 24, 1922]

acidity was developed by Penicillium when sugar was added to the decoction, but that alkalinity was developed by the same organism when no sugar was added. Working with four wood-rotting fungi, Meacham (7) found that these organisms grew over a large range of acidity and that a rather high concentration of the hydrogen ion is required to inhibit their growth. He places the limiting acidity at a p_H of about 1.7. His graph shows that in proceeding towards a more acid reaction, growth is fairly uniform until a p_H of about 2.6 is reached, when growth begins to decrease very rapidly to p_H 1.9. It then decreases more gradually to p_H 1.7. He states that frequently there is a maximum of growth which occurs at about p_H 3.

Brightman, Meacham, and Acree (8) in their investigations of "salt effects" and phenolsulphonphthalein indicators illustrate graphically some data obtained by Meacham on the growth of *Endothia parasitica* at various hydrogen-ion concentrations on bean decoction buffered with dipotassium hydrogen phosphate and acetic acid. As drawn, their curve for the initial p_H of the medium plotted against growth increases to a maximum at about p_H 4.5, falls off to about p_H 5.0, and then continues practically horizontally to p_H 8.0 where it descends rapidly to p_H 8.5. If this curve were redrawn through the points as plotted following the points more closely between p_H 4.0 and p_H 6, it would be seen that the maximum at p_H 4.5 is followed by a distinct minimum and a second maximum. Similarly, the curve of the final p_H of the medium plotted against the growth shows slight evidence of a minimum. Attention is drawn to this minimum, apparently overlooked by these workers, because of certain data presented in this paper which show a minimum in the growth of *Gibberella Saubinetii* as plotted against hydrogen-ion concentration.

Meacham, Hopfield, and Acree (9) grew *Endothia parasitica* on various media in which the hydrogen-ion concentration was regulated by means of phosphate-acetate and phosphate-phthalate buffer mixtures. They found that the organism grew well at a p_H of about 5.7.

The above named organisms all apparently have a rather wide range of acidity at which they will grow. In the case of the potato-scab organisms, however, Gillespie (10) found that the growth is inhibited by a relatively low acidity. All the strains he used showed inhibition at p_H 5.1, and usually there was no growth at all at p_H 4.8. Waksman (11) found that the limiting concentrations for the genus Actinomyces as a whole are about p_H 5.0 and p_H 9.0, although several species are able to grow at higher hydrogen-ion concentrations (p_H 4.6–4.8). The optimum reaction is placed at p_H 7.0–7.8.

Zeller, Schmitz, and Duggar (12) conclude from their work on wood-destroying fungi on liquid media that the hydrogen-ion concentration is not a limiting factor in the growth of these organisms. Webb (13) presents some interesting data on the relation of hydrogen-ion concentration to the germination of the spores of certain fungi. He determined the maximum and limiting acidities for the germination of the spores of *Aspergillus niger*, *Penicillium cyclopium*, *Botrytis cinerea*, and a species of Fusarium. His results with *Fusarium* sp. seem especially significant in connection with the data presented in this paper. Webb found with the spores of this fungus that at 22° after 20 hours no germination occurred at p_H 2.8, slight germination at 3.1, and increasing germination up to 5.0. From this point the germination falls off to 6.2, rises to a second high point at 7.4, and finally declines to 10.0. The curve therefore has two maxima with a minimum between. Webb's curve for the germination of the spores of *Fusarium* sp. at 27° also shows a distinct minimum at p_H 6.2. He also obtained minimum points, though less marked, in the germination curves of the spores of *Aspergillus niger* at 27° C. and of *Penicillium cyclopium* at 27° and 31° C.

In the present study, the effect of hydrogen-ion concentration on the growth of *Gibberella Saubinetii*, the causal organism of wheat scab, was determined. An authentic culture of the pathogene was secured from Professor J. G. Dickson of the University of Wisconsin. A single spore isolation from this culture was used throughout the studies here presented. Three experiments were performed. In the first the reaction of a liquid medium was adjusted with sulphuric acid and sodium hydroxide, in the second with phosphates, phosphoric acid, and potassium hydroxide, and in the third lactic acid was used with potato-dextrose agar.

Experiment 1. Growth on Liquid Media—Sulphuric-Acid-Sodium-Hydroxide Series

The culture solution used had the following composition:

KNO_3	2.0 g.
$MgSO_4.7H_2O$	0.5 g.
KH_2PO_4	0.1 g.
Glucose	10.0 g.
H_2O	1,000 cc.

Fifty cubic centimeters of this solution were added to each culture flask. Erlenmeyer Pyrex flasks of 150-cubic-centimeter capacity were used. The flasks containing the solution were sterilized, and the reaction was adjusted when they were cool by adding a varying number of drops of sterile solutions of $N/1$ and $N/10$ H_2SO_4 and $N/1$ and $N/10$ NaOH. Five cubic centimeters of the solution were then withdrawn aseptically from each flask for the determination of the hydrogen-ion concentration. The colorimetric method devised by Gillespie (2) was used in obtaining the p_H values. The cultures were inoculated with mycelium cut from potato-agar plates. They were incubated in a dark room at a temperature of 26° C. At the end of seven days, ten cubic centimeters of concentrated hydrochloric acid were added to each flask to stop the growth. The dry weights of the mycelial mats were determined by filtering into a Gooch crucible and drying at 110° C. It was found that the filtering through the Gooch filters was greatly facilitated by the addition of 40 to 50 cubic centimeters of 95 percent alcohol to the contents of the flask. The mats were washed five or six times with 50 percent alcohol after being thrown on the filter. The results of this experiment are presented in table 1.

TABLE 1. *Hydrogen-ion Concentration and Growth of Gibberella Saubinetii in Liquid Media in which the Reaction was Adjusted with Sulphuric Acid and Sodium Hydroxide. Temperature, 26° C.*

p_H (Before)	p_H (After)	p_H (Average)	Milligrams Dry Weight
3.85	4.4	4.12	1.6
4.8	3.6	4.2	30.4
5.37	4.5	4.44	54.2
4.8	6.0	5.4	48.0
5.5	5.5	5.5	60.8
5.8	6.2	6.0	60.0
7.5	5.2	6.3	34.8
7.5	5.2	6.35	27.0
7.4	5.6	6.5	36.8
7.5	5.7	6.6	46.8
7.6	5.9	6.75	50.4
....	7.5	7.5	79.6
8.25	6.9	7.57	74.2

If the average hydrogen-ion concentration expressed as p_H is plotted on the abscissa and the dry weight of the fungus is plotted on the ordinate as in figure 1, it is seen that with decreasing acidity from $p_H = 4.0$ the growth of the fungus increases to a maximum at about 5.5 and then falls sharply to a minimum at 6.3. It then rises again to about 7.5.

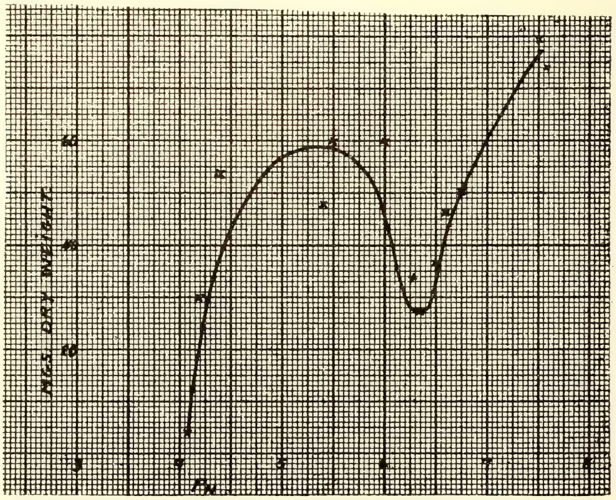


FIG. 1. Relation of hydrogen-ion concentration of liquid culture solution and growth of *Gibberella Saubinetii*—sulphuric-acid-sodium-hydroxide series.

Experiment 2. Growth on Liquid Media—Phosphate Series

In order to check the result reported above, a similar experiment was performed using phosphate solutions to adjust the reaction. Three periods of growth were used in this case instead of one. Four, seven, and fourteen days were selected as suitable lengths of time. At the end of each of these periods a series of cultures was removed and the p_H values and dry weights of the mycelium were determined as before.

The culture solution used contained in one liter:

KNO_3	2.0 g.
$MgSO_4.7H_2O$	0.5 g.
Glucose	10.0 g.

In addition, the solution designated as the "acid solution" contained 9.077 g. KH_2PO_4 per liter, and the "basic solution" contained 11.616 g. of K_2HPO_4 per liter. This made two culture solutions of $M/15$ concentration as regards the acid and basic phosphate respectively. By mixing these two solutions in varying proportion the hydrogen-ion concentrations desired could be obtained. This was roughly accomplished by using the titration curve of Sörensen (14) for phosphate buffer mixtures. For more acid or more alkaline mixtures than could be obtained in this way, $M/15$ concentrations of H_3PO_4 and KOH were prepared in culture solution and added in varying proportions to the "acid" or "basic" solution respectively.

TABLE 2. *Hydrogen-ion Concentration and Growth of Gibberella Saubinetii in Liquid Media in which the Reaction was Adjusted with Primary Potassium Phosphate, Secondary Potassium Phosphate, Phosphoric Acid, and Potassium Hydroxide*

Results after 4 Days		Results after 7 Days		Results after 14 Days	
p_H	Dry Weight (Mg.)	p_H	Dry Weight (Mg.)	p_H	Dry Weight (Mg.)
2.8	0.0	2.77	0.3	2.8	2.2
2.9	0.6	2.9	0.8	3.15	3.6
3.05	0.0	3.1	1.8	3.25	17.2
3.4	1.8	3.47	3.8	3.85	70.6
4.25	6.0	4.6	47.4	5.0	42.2
4.75	4.8	4.6	23.4	5.4	70.4
5.35	5.4	5.07	20.2	5.6	72.2
5.7	7.0	5.45	15.2	5.75	67.8
6.25	9.2	6.25	14.4	5.75	75.0
6.95	3.0	6.85	28.2	7.15	71.1
7.1	7.0	7.1	48.8	7.45	66.1*
7.1	10.6	7.15	31.7[1]	7.55	73.2
7.1	2.8	7.2	13.6	7.7	47.8
7.15	7.8	7.2	3.2	7.75	70.8
7.15	0.8	7.4	2.2		
7.2	12.4	7.4	2.2		
7.2	3.0				

* Average of three determinations.

Inoculation and dry-weight determinations were carried out as before. The cultures were incubated at a temperature of 25.8° C. In table 2 the results at the end of four, seven, and fourteen days are shown. The p_H values are the averages of determinations made at the beginning and end of the period.

The results are expressed graphically in figures 2, 3, and 4. In general the curves are similar in form to the one obtained in the first experiment.

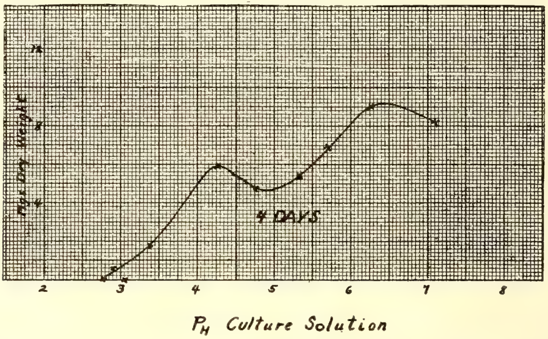


FIG. 2. Hydrogen-ion concentration and growth of *G. Saubinetii* on liquid media—phosphate series after four days.

At the end of four days there is a definite minimum in the curve. After seven days the depression in the curve is more marked, with its lowest point at p_H from 5.5–6.0. In the most acid cultures slight indications of growth were noticed at this time, but the first culture showed no appreciable dry weight. It is interesting to note that in the most acid cultures there were formed a number of colonies, which fact would indicate a stimulation of

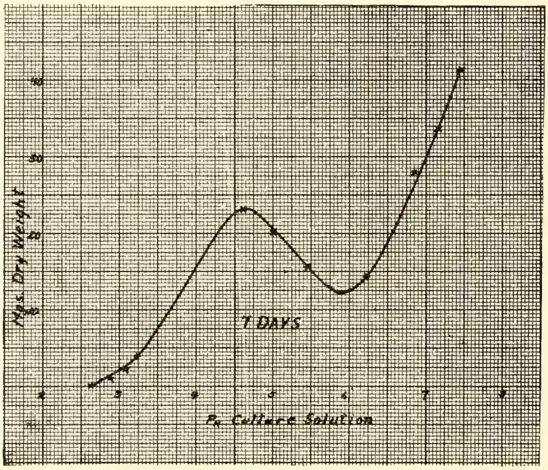


FIG. 3. Hydrogen-ion concentration and growth of *G. Saubinetii* on liquid media—phosphate series after seven days.

conidial production or perhaps segmentation of the mycelium. In the less acid cultures a single colony was usually formed from the original inoculum.

At the end of the fourteen-day period (fig. 4) a depression is still present but it is not so noticeable. At this time the growth curve resembles somewhat the growth curve obtained by Meacham (7) with wood-rotting fungi.

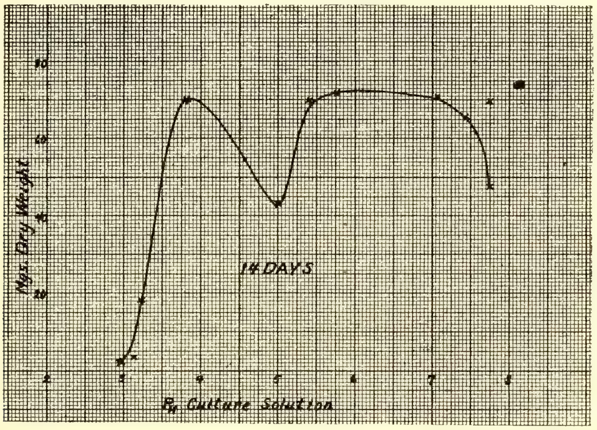


FIG. 4. Hydrogen-ion concentration and growth of *G. Saubinetii* on liquid media—phosphate series after fourteen days.

Experiment 3. Growth on a Solid Medium—Lactic-Acid Series

The growth of the organism was also studied on potato-dextrose-agar plates of varying hydrogen-ion concentration. Lactic acid was used in adjusting the reaction as follows: to 20-cubic-centimeter portions of the melted medium a varying number of drops of lactic acid (50 percent by volume) was added and petri dishes were poured. A separate series of tubes was prepared in the same manner and used for the hydrogen-ion determinations. Each plate was inoculated in five or six places with the mycelium of *Gibberella Saubinetii* on small blocks of agar, and the plates were incubated at 25° C. At the end of 21 and 44 hours respectively, measurements of the diameter of the colonies in millimeters were made. The results at the ends of these two periods are shown in table 3 and represented graphically in figure 5. The diameter given is the average diameter of all the colonies on a plate.

TABLE 3. *Hydrogen-ion Concentration and the Growth of Gibberella Saubinetii on Potato-Dextrose Agar in which the Reaction was Adjusted by Means of Lactic Acid*

Drops of Lactic Acid per 20 Cc. of Medium	P_H	Average Diameter of Colonies after 21 Hours (Mm.)	Average Diameter of Colonies after 44 Hours (Mm.)
0	6.9	18.2	41.2
1	4.5	13.7	32.6
2	4.0	10.4	25.6
3	3.8	9.4	21.0
4	3.6	1.7	10.6

It will be noticed by referring to figure 5 that there is no depression in this curve. This is due to the fact that no acidities intermediate between p_H 4.5 and p_H 6.9 were used. The points at which the minimum occurs were not present in this series, and therefore no minimum was evident. Another series with potato agar including acidities between p_H 4.5 and 7.0 demonstrates that a minimum also occurs on potato-dextrose agar. In order to obtain these intermediate values, a solution of lactic acid one eighth the strength of that employed in the former experiment was used. The data for this experiment are given in table 4 and represented graphically in figure 6.

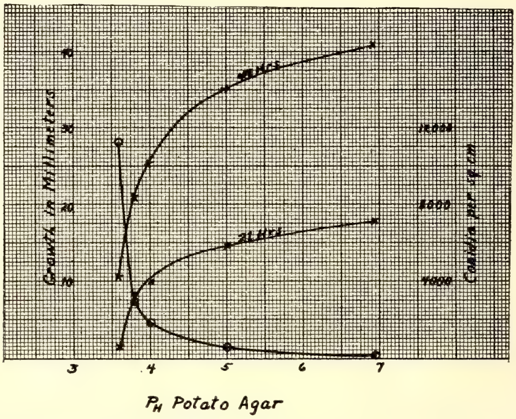


FIG. 5. Hydrogen-ion concentration and growth and conidial production in *G. Saubinetii* on potato-dextrose agar—lactic-acid series.

TABLE 4. *Hydrogen-ion Concentration and Growth of Gibberella Saubinettii on Potato-dextrose Agar in which the Reaction was Adjusted by Means of Lactic Acid*

Drops of 50 Percent Lactic Acid per 20 Cc. of Medium	p_H	Average Diameter of Colonies after 19 Hours (Mm.)	Average Diameter of Colonies after 24 Hours (Mm.)	Average Diameter of Colonies after 43 Hours (Mm.)
0	7.3	12.42	17.06	34.26
1/16	7.0	13.86	18.00	34.26
1/8	6.0	14.12	17.32	32.60
2/8	5.7	12.00	15.94	31.30
3/8	5.2	10.50	14.92	30.64
4/8	5.0	12.48	16.28	30.72
6/8	4.65	11.04	14.40	28.48
1	4.4	10.64	13.66	25.74
2	4.0	9.38	11.60	21.00
3	3.8	7.70	9.64	17.30
4	3.6	5.60	7.48	13.78
5	3.5	3.70	5.14	10.50

From the curve in figure 6 it can be noted that a depression in the growth-acidity curve similar to those on liquid media is obtained on potato-dextrose agar. The deepest minimum appears after 19 hours. After 43 hours there is but a slight depression. The minima appear at a p_H of 5.0–5.5.

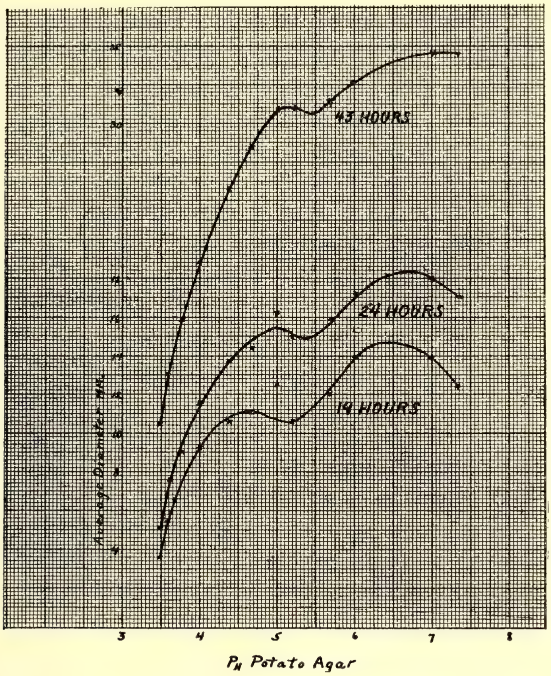


FIG. 6. Hydrogen-ion concentration and growth of *G. Saubinetii* on potato-dextrose agar after nineteen, twenty-four, and forty-three hours—lactic-acid series.

THE RELATION BETWEEN THE HYDROGEN-ION CONCENTRATION OF THE SOIL AND SEEDLING INFECTION OF WHEAT BY GIBBERELLA SAUBINETII

Gillespie and Hurst (3, 4) have investigated the acidity of soils in which potato scab was prevalent and of those relatively free from scab. They found the mean value of the exponent for the Washburn type of soil, on which scab is prevalent, to be p_H 5.93, while that of the Caribou type, which is generally free from scab, was p_H 5.2. This agrees with the results of Gillespie (10), previously discussed, that the growth of the potato-scab organism is inhibited at hydrogen-ion concentrations between p_H 5.2 and p_H 4.8.

More recently Martin (15, 16), in working on the effect of inoculated and uninoculated sulphur on the development of potato scab, determined as well the effect of the sulphur on the hydrogen-ion concentration of the soil. He found that increasing the amounts of sulphur applied to the soil increased the hydrogen-ion concentration of the soil and correlated this increase with the decreasing percentage of scabby tubers. The increase in acidity is due to the oxidation of the sulphur to sulphuric acid. He ascribes the advantage of the inoculated sulphur to its more rapid conversion to the acid.

In the experiments reported in this paper, the effect of hydrogen-ion concentration upon seedling infection of wheat by *Gibberella Saubinetii* is reported. The experiments were carried out under greenhouse conditions in soil in flats. The reaction of the soil was adjusted by means of sulphuric or hydrochloric acid and sodium hydroxide.

Experimental

Soil-Acidity Determinations. These were made according to the method of Gillespie (2). The soils were air-dried and passed through a millimeter sieve. Thirty grams of this soil were placed in a 100-cubic-centimeter centrifuge tube with 30 cubic centimeters of water, the top was closed with the palm of the hand, and the mixture was shaken violently about fifty times. The tube was then centrifuged for about fifteen minutes, and the acidity was determined colorimetrically in the supernatant liquid by means of Gillespie's drop-ratio method. Determinations were checked when possible by using more than one indicator. Gillespie's standards were checked against standard phosphate, phthalate, and acetate buffer mixtures, which were in turn checked by electrometric measurements. The agreement was to 0.1 of a p_H in all cases.

Experiment 4. To a rich loamy soil about one fourth sand was added and the whole was well mixed. Eighteen kilograms were then weighed into each of nineteen flats. The dimensions of the flats were 12 x 18 x 6 inches. A sample of the soil was divided into 250-gram portions, and to each was added a given amount of $N/1$ H_2SO_4 or $N/1$ NaOH made up to a volume of 40 cubic centimeters with water. After mixing the soil and the acid or alkali well and preparing a sample as described above, the hydrogen-ion concentration was determined as described above. From these data a rough preliminary titration curve was constructed. From this curve desired values could be obtained to use in adjusting the reaction of the larger portions of soil. The original soil had a p_H of 5.9. The effects of the acid and alkali on the soil in the flats are shown in table 5.

TABLE 5. *The Effect of Sulphuric Acid and Sodium Hydroxide on the Reaction of the Soil Used in Experiment 4*

Treatment	Cc. $N/1$ H_2SO_4 per 250 G. Soil								Cc. $N/1$ NaOH per 250 G. Soil									
	25	20	15	10	7	3	2	1	0	1	2	5	7	10	15	20	25
p_H	3.4	3.6	3.8	4.4	4.6	5.3	5.5	5.6	5.9	6.4	6.6	6.9	7.6	7.9	8.3	8.6	9.0

The data given in table 5 are also presented in the form of a graph (fig. 7), which is a titration curve of this soil. The acid or alkali added to the flats was thoroughly mixed with the 18 kilograms of soil so that a uniform acidity would be obtained. The soil in seventeen of the flats was adjusted in this manner, two others being retained as checks.

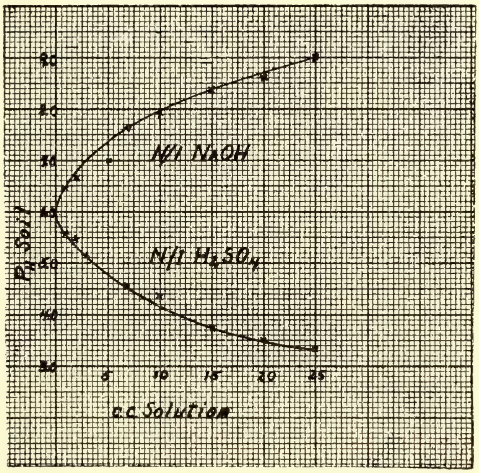


FIG. 7. Titration curve of soil used in experiment 4 with $N/1$ NaOH and $N/1$ H_2SO_4.

The flats were allowed to stand for about a week, when soil samples were taken for acidity determinations. A cork borer was used in taking the sample, and several "cores" of soil were taken from various parts of the flat. They were thoroughly mixed to insure a representative sample. At the end of a week the flats were planted with wheat.

A good sample of Fultz wheat was used. The seed, with the exception of that used in the two control flats, was shaken before planting with a spore suspension of *Gibberella Saubinetii* from a single-spore culture. Approximately 80 seeds were planted in each flat, in two-inch checks, two seeds per hill. The surface of the soil was then sprayed with a spore suspension. A fairly uniform moisture content was maintained by watering the flats with a fine spray from a garden hose. Observations were made of the soil temperature at frequent intervals. This averaged about 20° C. and varied from 20–25° C. A set of soil samples for hydrogen-ion determinations was taken at intervals of one week, four samples in all being taken.

TABLE 6. *The Effect of Soil Reaction on the Germination of Wheat Seedlings*

No. of Seedlings up in 4 Days	3	16	32	33	31	41	24	39	29	38	49	55	34	34	10	0	0
PH	3.65	3.8	4.25	4.55	4.75	5.4	5.6	5.7	5.9	6.4	6.6	7.15	7.45	7.75	8.3	8.65	9.0

Seedlings began to appear in some of the flats at the end of three days, and at the end of four days the number of seedlings in each flat was counted. The number of seedlings with the corresponding p_H values is shown in table 6.

In figure 8 the data of table 6 are shown graphically. This curve is fairly uniform and shows a marked depression at a p_H of about 5.5 with two maxima on either side, one at a p_H of about 4.5 and the other near neutrality (p_H = 7.0).

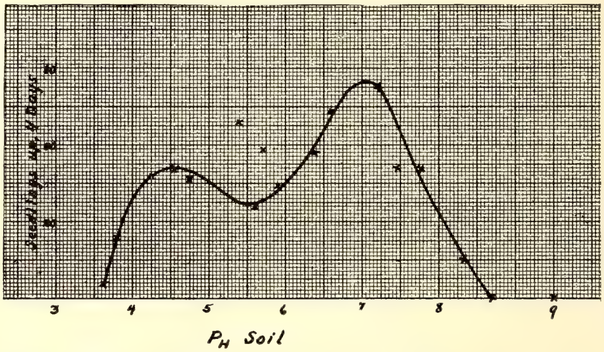


FIG. 8. Relation of number of seedlings up in four days and hydrogen-ion concentration of soil.

It is not certain whether the variations in germination noted in the data in table 6 and in figure 8 are due entirely to the effect of variations in the hydrogen-ion concentration upon infection by *Gibberella Saubinetii*. From experiment 5, however, and also from unpublished data secured by Dr. W. J. Robbins, it is very probable that, apart from infection, variations in the hydrogen-ion concentration cause the curve of the germination of wheat to pass through two maxima with a minimum between.

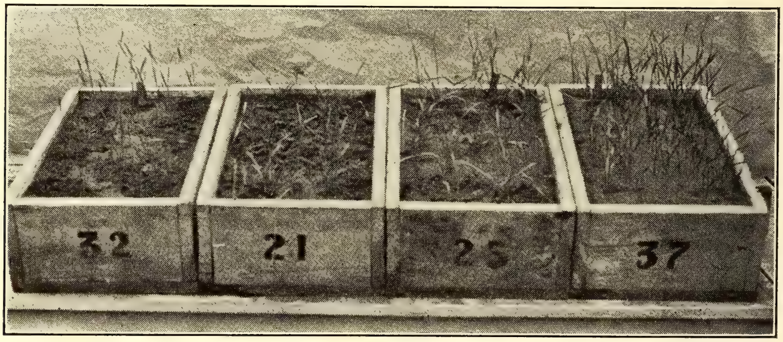


FIG. 9. Soil acidity and seedling infection—the three most acid flats of soil, on left, compared with check flat on right; p_H 3.6, p_H 3.9, p_H 4.4, check.

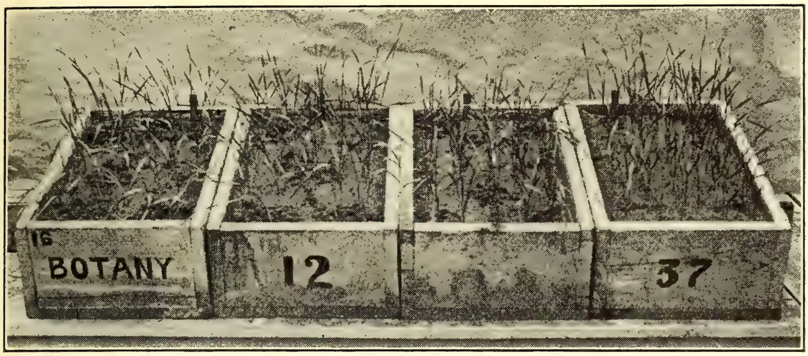


FIG. 10. Soil acidity and seedling infection—p_H 4.65, p_H 4.8, p_H 5.5, check.

Infection in the seedlings was first observed ten days after planting, and frequent counts were made of the total number of seedlings as well as of those known to be infected. Figures 9 to 13 show the appearance of the seedlings as contrasted with one of the uninoculated, untreated control flats. The thinness of stand and the wilting of the plants in the first three flats (the most acid) show a striking contrast to the condition of the control. At a p_H of 5.5 the stand is more nearly perfect, but as we approach the more alkaline soils the stand again becomes poor until at a $p_H = 9.0$ there are no seedlings up.

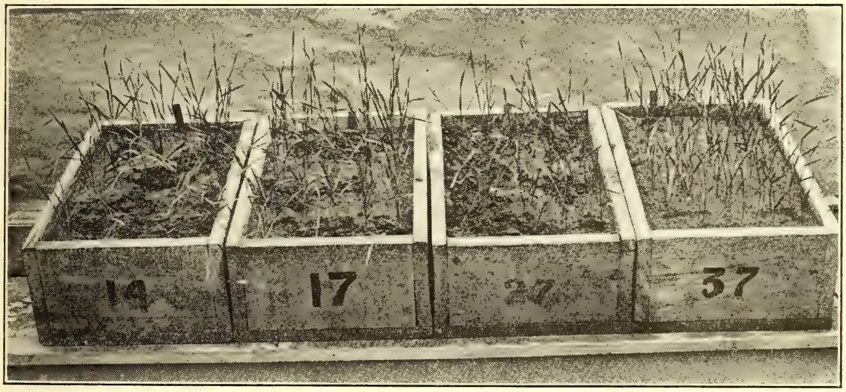


FIG. 11. Soil acidity and infection—p_H 5.7, p_H 5.8, p_H 5.9, check.

At the end of three weeks all seedlings were removed and the number of infections was recorded. Many seedlings which appeared healthy were found to be infected below the surface of the soil. In the most acid and most alkaline soils many seedlings which had germinated were rotted before reaching the surface of the soil. In most cases the greater amount of injury was due to stem rot, although in the very alkaline soils more root rot was observed.

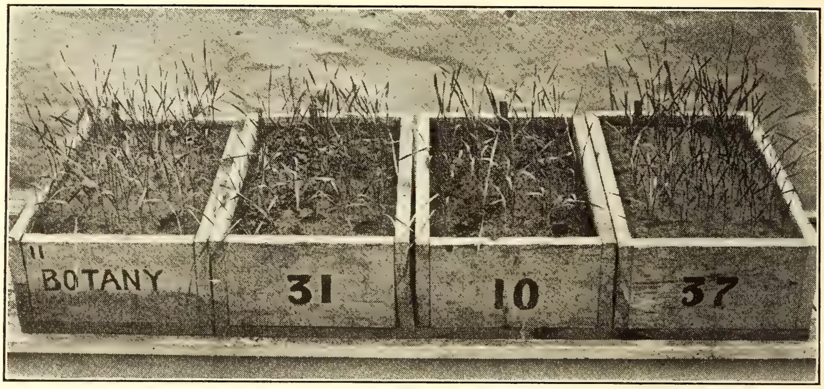


FIG. 12. Soil acidity and infection—p_H 7.2, p_H 7.5, p_H 7.8, check.

Re-isolations were made from several typical lesions from each flat, and pure cultures of *Gibberella Saubinetii* were invariably obtained. The two control flats containing the original uninoculated soil showed a perfect stand and no diseased seedlings. In table 7 a summary of the data is presented. These data are also shown graphically in figure 14. For an experiment of this type the curve is very uniform. A maximum appears at $p_H = 4.0$, followed by a clear-cut minimum at $p_H = 5.5$. As the more alkaline soils are reached the curve rises, and in the most alkaline infection is as high as 100 percent.

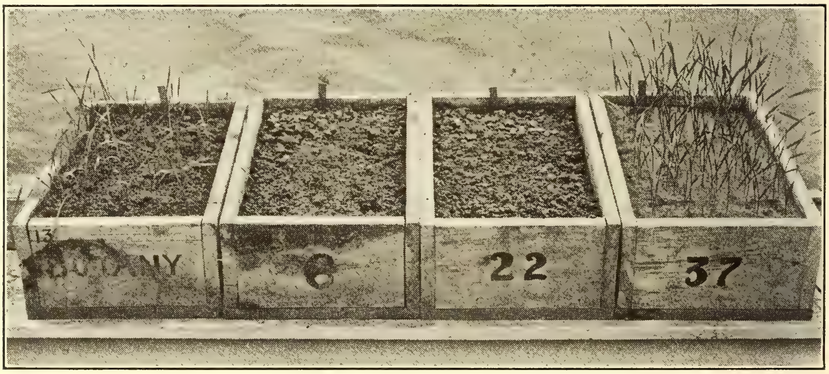


FIG. 13. Soil acidity and infection—p_H 8.2, p_H 8.6, p_H 9.0, check.

Experiment 5. In the above described experiment a control or uninoculated soil was not used for each soil acidity, and the depression in the curve might be caused by the sulphate ion. A second experiment was therefore performed in which hydrochloric acid was used in place of sulphuric acid in adjusting the soil reaction, and a flat of uninoculated soil at each acidity was used as a check.

TABLE 7. *Hydrogen-ion Concentration of the Soil and Seedling Infection. Sulphuric-acid Series*

No. of Flat	pH (Average)	Total Plants	Number Infected	Percent Infected
32	3.63	76	43	56.6
21	3.90	77	63	81.8
25	4.36	83	63	75.9
16	4.65	70	42	60.0
12	4.80	76	32	42.1
35	5.53	82	19	23.2
14	5.66	70	39	55.7
17	5.80	73	29	39.7
27	5.90	75	41	54.7
15	6.47	80	43	53.8
29	6.67	84	44	52.4
11	7.22	79	42	53.2
31	7.48	82	54	65.9
10	7.78	79	39	49.4
13	8.22	79	72	91.2
6	8.63	79	79	100.0
22	9.06	77	77	100.0
37	Control	80	0	0
7	Control	81	0	0

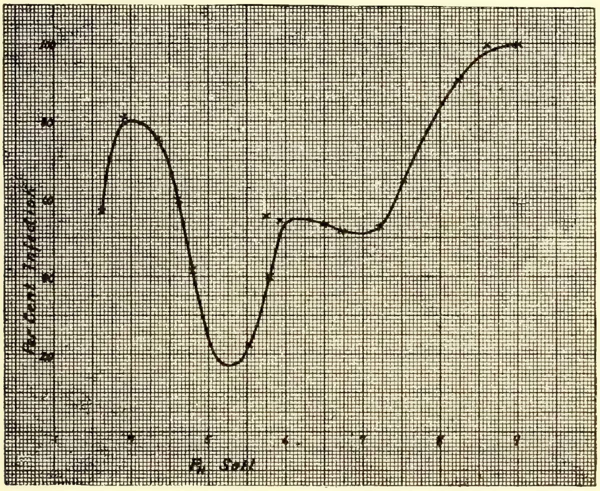


FIG. 14. Soil acidity and infection—hydrogen-ion concentration in its relation to the percentage of seedling infection—sulphuric-acid series.

Another soil sample was used. Its reaction was adjusted in essentially the same manner as before. Using 2*N* HCl and 2*N* NaOH, a titration curve of this soil was constructed and the quantities of acid or alkali required to secure the desired acidities were calculated for 36 kilograms of soil. After thorough mixing, the soil was divided between two flats, one to be inoculated and the other for a control. The titration data which are given in table 8 are the results obtained in the flats after mixing the 36 kilograms of soil in each case with the required amount of solution. The results are expressed, however, on the basis of 250 grams of soil. A curve constructed from these data is shown in figure 15.

TABLE 8. *The Effect of Hydrochloric Acid and Sodium Hydroxide on the Reaction of the Soil Used in Experiment 5*

Treatment	Cc. 2*N* HCl per 250 G. of Soil											Cc. 2*N* NaOH				
	13	10	7	5	4	3.5	3	2.5	2	1.5	1	0	2	5	10	13
p_H	3.5	3.8	4.47	4.25	5.12	5.3	5.43	5.47	5.47	5.6	5.74	6.4	6.98	7.18	7.83	7.83

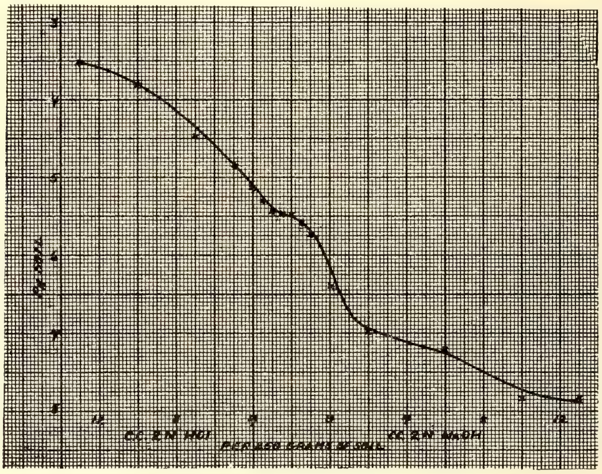


FIG. 15. Titration curve of soil used in experiment 5 with 2*N* HCl and 2*N* NaOH.

Inoculation and planting were carried out as in experiment 4. The seeds planted in the sixteen uninoculated flats were soaked in sterile water for the same length of time that the others were immersed in the spore suspension.

The appearance of the seedlings at the surface of the soil was first noted three days after planting. A larger number of seedlings was found in the inoculated series. In the latter series seventeen seedlings were counted, as compared with seven in the inoculated series. The total number of seedlings up in the uninoculated flats continued to be larger than in the inoculated set. The relation between the two series and the relation of each to the acidity are brought out in table 9 and also in the graphs in figures 16 and 17. At this time it is not certain whether or not this difference is due to infection by *Gibberella Saubinetii*. The p_H values given are the averages of two series of determinations made one day before and four days after this period respectively.

TABLE 9. *Relation of Soil Acidity to the Number of Seedlings Up in Four Days in the Inoculated and Uninoculated Series*

Seedlings up 4 Days Controls	0	0	0	1	4	12	1	2	4	16	29	57	53	38	5	9
Seedlings up 4 Days —Inoculated Series	0	0	0	0	0	0	2	4	0	15	21	26	27	30	1	0
p_H	3.42	3.8	4.35	4.56	4.89	5.25	5.37	5.4	5.47	5.57	5.71	6.3	6.91	7.25	7.8	7.82

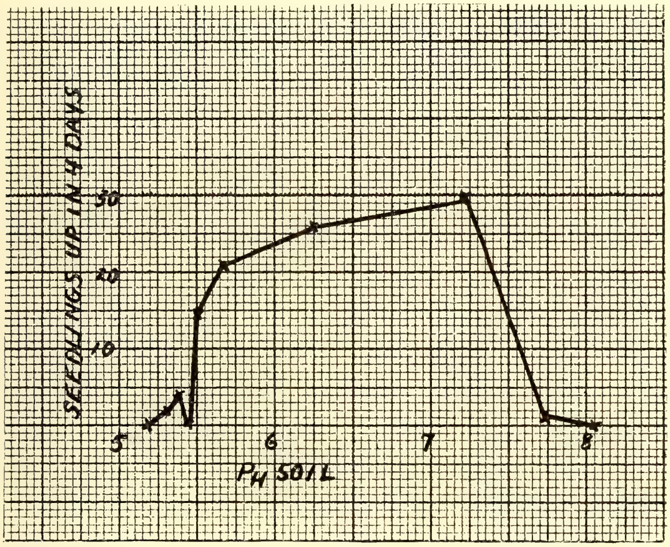


FIG. 16. Hydrogen-ion concentration of soil and number of seedlings up in four days in inoculated flats. Hydrochloric-acid series.

Although there is only slight evidence of a minimum in the inoculated series, due perhaps to unfavorable greenhouse conditions at the time this experiment was run, the uninoculated series shows a distinct minimum at about p_H 5.5. This indicates not only that the phenomenon of this minimum is related to infection, but that there is an independent action of the soil acidity on the germination of wheat.

The first signs of infection at the surface of the soil were noted nine days after planting. The final data were taken at the end of three weeks. The plants were all removed as in experiment 4, and the percentage of infection was determined. These data are presented in table 10 and are shown graphically in figure 18. The p_H values in this case are the averages of three series of determinations. The temperature during this experiment varied from about 12° C. to 30° C., with an average of about 19.5° C.

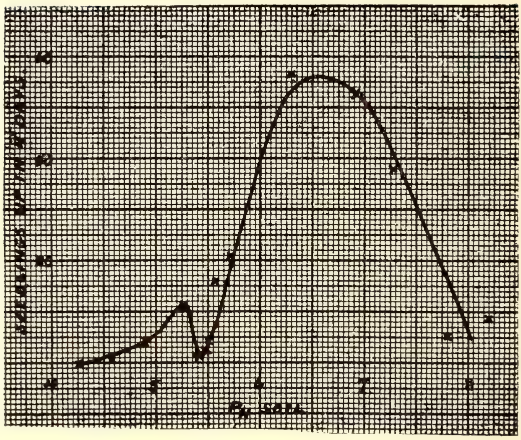


FIG. 17. Hydrogen-ion concentration of soil and number of seedlings up in four days in uninoculated flats. Hydrochloric-acid series.

TABLE 10. *Hydrogen-ion Concentration of the Soil and Seedling Infection. Hydrochloric-acid Series*

No. of Flat	p_H (Average)	Total Plants	Number Infected	Percent Infected
33	3.5	67	48	71.6
42	3.8	50	21	42.0
40	4.47	65	50	76.9
49	4.85	58	44	75.9
56	5.12	62	37	59.7
41	5.3	61	39	64.0
38	5.43	66	31	47.0
55	5.47	69	28	40.5
28	5.47	63	22	34.9
45	5.6	70	25	35.7
18	5.74	78	32	41.0
24	6.4	79	38	48.1
1	6.98	80	39	48.7
3	7.18	75	40	53.3
46	7.83	58	45	77.6
43	7.83	69	52	75.3

In the uninoculated flats an occasional infected seedling was found, due perhaps to natural infection from the seed. All others were uninfected at the time of digging. Examining the data given in table 10 as shown in figure 18, it can be seen that when hydrochloric acid is used in place of sulphuric acid in the adjustment of the soil to various acidities, the same phenomenon occurs as was noted in experiment 4. A minimum in the infection is present at a p_H of 5.5. This is strong evidence that the minimum in the infection curve is due to the hydrogen-ion concentration alone. This is strengthened of course by the results obtained in the growth of *Gibberella Saubinetii*, when an adjustment of the reaction with sulphuric acid, acid phosphate, and lactic acid gave similar low points in the growth-acidity curve.

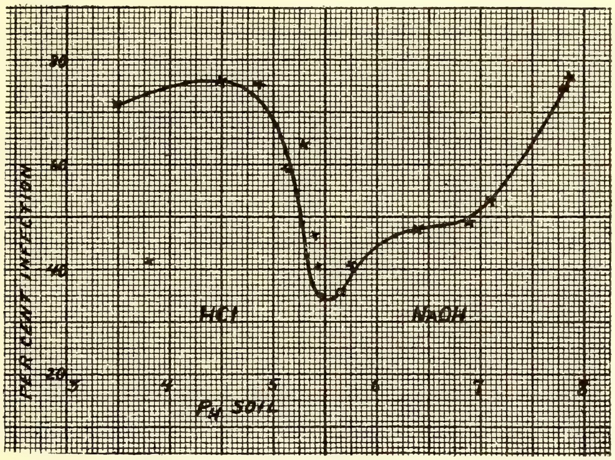


FIG. 18. Soil acidity and infection—hydrogen-ion concentration in its relation to the percentage of seedling infection. Hydrochloric-acid series.

SUMMARY

The experiments reported in this paper were undertaken to determine the relation of hydrogen-ion concentration to wheat scab. It was desired to find if possible a limiting acidity for seedling infection of wheat by the causal organism of this disease, *Gibberella Saubinetii.*

A study of the pathogene *Gibberella Saubinetii* shows that, although a wide range of acidity is tolerated, there is a minimum in the growth curve. This minimum in the curve was found to be present in three different series of cultures. In the first series the reaction of a liquid medium was adjusted by means of sulphuric acid and sodium hydroxide; in the second, primary and secondary potassium phosphate, phosphoric acid, and potassium hydroxide were used in a liquid medium, and in the third, the acidity of potato-dextrose agar was varied by means of lactic acid. The minimum point in the curve varied from about p_H 5.5 to p_H 6.0. This is similar to the results of Webb (13) in his work on spore germination and hydrogen-ion concentration. The use of various substances to change the reaction shows that the effect on the growth is due to the hydrogen-ion concentration and not to other molecules or ions.

An interesting correlation appears in the relation of soil acidity to seedling infection. Here also a minimum was obtained in two cases at p_H 5.5. In one instance the reaction was adjusted by means of sulphuric acid and sodium hydroxide, and in the other hydrochloric acid and sodium hydroxide were used. It seems, therefore, that there is a relation between the effect of acidity on the growth of the pathogene and its effect on infection. Furthermore, it appears from results obtained on the effect of acidity on the rate of germination of wheat in the control flats, which also shows a minimum, that there is as well an effect of the hydrogen ion on the host, which causes a depression in the infection curve. How this comes about is not certain, but it seems plausible that both these phenomena affect the severity of the infection. Nor is it at all clear at present what the cause of the depression in these curves is. In the opinion of the writer this will be solved only by further study of hydrogen-ion concentration in its relation to other factors.

Because of the great importance not only of wheat scab, but of fusarial diseases as a whole, it is hoped that the results obtained in this study may have a practical bearing in the control of such soil-borne pathogens. Attention is directed to the fact that the minimum in the infection curve occurs at p_H 5.5, a not unusual soil reaction to which the soil could easily be adjusted.

The writer wishes to express his thanks to Dr. W. J. Robbins for material assistance and helpful advice during the progress of the work.

Department of Botany,
University of Missouri

LITERATURE CITED

1. **Gillespie, L. J.** The reaction of soil and measurements of hydrogen-ion concentration. Jour. Wash. Acad. Sci. **6**: 7–16. 1916.
2. ——. Colorimetric determination of hydrogen-ion concentration without buffer mixtures, with especial reference to soils. Soil Sci. **9**: 115–136. 1920.
3. ——, **and Hurst, L. A.** Hydrogen-ion concentration measurements of soils of two types: Caribou loam and Washburn loam. Soil Sci. **4**: 313–319. 1917.
4. —— ——. Hydrogen-ion concentration—soil type—common potato scab. Soil Sci. **6**: 219–236. 1918.
5. **Clark, W. M., and Lubs, H. A.** The colorimetric determination of hydrogen-ion concentration and its applications in bacteriology. Jour. Bact. **2**: 1–34, 109–136, 191–236. 1917.
6. **Duggar, B. M., Severy, J. W., and Schmitz, H.** Studies in the physiology of the fungi IV. The growth of certain fungi in plant decoctions, preliminary account. Ann. Mo. Bot. Gard. **4**: 165–173. 1917.
7. **Meacham, M. R.** Note upon the hydrogen ion concentration necessary to inhibit the growth of four wood-destroying fungi. Science n. s. **48**: 499, 500. 1918.
8. **Brightman, C. L., Meacham, M. R., and Acree, S. F.** A spectrophotometric study of the "salt effects" of phosphates upon the color of phenolsulphonphthalein salts and some biological applications. Jour. Bact. **5**: 169–180. 1920.
9. **Meacham, M. R., Hopfield, J. H., and Acree, S. F.** The relative effect of phosphate-acetate and phosphate-phthalate buffer mixtures upon the growth of *Endothia parasitica* on malt extract and corn meal media. Jour. Bact. **5**: 305–308. 1920.
10. **Gillespie, L. J.** The growth of the potato scab organism at various hydrogen-ion concentrations as related to the comparative freedom of acid soils from the potato scab. Phytopath. **8**: 257–269. 1918.
11. **Waksman, S. A.** Cultural studies of species of Actinomyces. Soil Sci. **8**: 71–207. 1919.
12. **Zeller, S. M., Schmitz, H., and Duggar, B. M.** Studies in the physiology of fungi VII. Growth of wood destroying fungi on liquid media. Ann. Mo. Bot. Gard. **6**: 137–142. 1919.

13. **Webb, R. W.** Studies in the physiology of fungi X. Germination of the spores of certain fungi in relation to hydrogen ion concentration. Ann. Mo. Bot. Gard. **6**: 201–222. 1919.

14. **Sörensen, S. P. L.** Enzymstudien II. Über die Messung und die Bedeutung der Wasserstoffionenkonzentration bei enzymatischen Prozessen. Biochem. Zeit. **21**: 131–200, 201–304. 1909.

15. **Martin, W. H.** The relation of sulphur to soil acidity and to the control of potato scab. Soil Sci. **9**: 393–409. 1920.

16. ——. A comparison of inoculated and uninoculated sulphur for the control of potato scab. Soil Sci. **11**: 75–84. 1921.

ON THE PHYSIOLOGICAL BALANCE IN NUTRIENT SOLUTIONS FOR PLANT CULTURES

W. F. Gericke

(Received for publication July 11, 1921)

Wheat seedlings grown for one day in a dilute solution of KNO_3, the next day in one of $MgHPO_4$, the third day in one of $CaSO_4$, and these changes continued in rotation at twenty-four-hour intervals for four weeks, grew as large and produced approximately as much dry weight as those grown in complete nutrient solutions of the above-named salts and in other three-salt solutions that supplied the same ions. The plants grown in these three single-salt solutions were also much superior to those grown in other types of single-salt solutions that were changed likewise at twenty-four-hour intervals. Altogether, ten different simple nutrient salts were used in the tests, and these were selected to give six different types of nutrient solutions; three or four salts being required for each type, which, with a trace of iron salt added, supplied the essential salt or mineral constituents for plant growth in aqueous solutions. All solutions, with the exception noted below, were of approximately the same osmotic value, this being approximately equal to one atmosphere osmotic pressure. Each single-salt solution, aside from the trace of iron it contained (this being added as $FeSO_4$), supplied the plants with two essential nutrients, both cation and anion of the salts used being of those atoms or groups of atoms considered necessary for plant growth. It required three days to make one rotation to bring the plants in contact with the three nutrient solutions of their respective types, which supplied them with all the essential elements in nutrient solutions, *viz.*: NO_3, SO_4, PO_4, K, Ca, Mg, and Fe.

The salts used for the different types[1] were as follows:

Type I, KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$; type II, K_2SO_4, $Ca(NO_3)_2$, $MgHPO_4$; type III, KNO_3, $CaHPO_4$ (saturated solution plus enough $Ca(H_2PO_4)_2$ to give together 0.2 atmosphere osmotic value), $MgSO_4$; type IV, K_2SO_4, $CaHPO_4$ (saturated solution plus enough $Ca(H_2PO_4)_2$ to give together 0.2 atmosphere osmotic value), $Mg(NO_3)_2$; type V, KNO_3, $CaSO_4$, $MgHPO_4$; type VI, KH_2PO_4, $CaSO_4$, $Mg(NO_3)_2$.

The change of the cultures from one solution to another successively within each of the several types at twenty-four-hour intervals covered the possibilities respecting order of rotation. All cultures were rinsed with distilled water before being transferred to another solution. The cultures were in contact with the solutions belonging to a type for an equal length of time. The experiment was planned to supply the plants with the nutrients in piecemeal fashion, and not together at one time, as was the case with those grown in complete nutrient solutions.

---

[1] For the first mention of these types, see Livingston, B. E., and Tottingham, W. E. A new three-salt nutrient solution for plant cultures. Amer. Jour. Bot. 5: 337–346. 1918. Types II, III, IV, and V were modified by the author by substituting $CaHPO_4$ for $Ca(H_2PO_4)_2$ and $MgHPO_4$ for $Mg(H_2PO_4)_2$, the primary phosphate salts in the original being too acid for these tests.

It was conceived as very probable that some of the nutrient salts would be found better suited for plant growth than others, because of differences in properties due to the composition of the salts. The results of the tests are given in table 1.

TABLE 1. *Dry Weight in Grams of Wheat Seedlings Grown Four Weeks in Six Different Types of Single Salt Solutions and in Complete Nutrient Solutions*

(Averages of 24 cultures of 5 plants per culture for each type and for the six types of complete nutrient solutions)

Type I	Type II	Type III	Type IV	Type V	Type VI	Complete Solutions (All Types)
.51	.61	.59	.40	.96	.43	1.02

The table shows that the salts of type V, that is, KNO_3, $MgHPO_4$, and $CaSO_4$, when used singly gave far better yields than did those of any other type. They were approximately as good as those of any type of complete nutrient solution tested, in which the total osmotic value was divided equally among three salts. As to the order of change of cultures from one solution to another, this appeared to be of only minor importance, and hence no data on this matter need be given; but the kind of salt used in making the changes was of great importance. While the p_H values of the single-salt solutions employed were not the same in all cases, nevertheless they were within the range appropriate to good plant growth in complete solutions, and it is, therefore, assumed that the differences in dry weight produced were due to other causes than reaction of solution. Because only one salt was used at a time, it appears that the cause must be in the way in which two essential ions are paired, that is by the composition of the salt. Observations of the tests and the interpretation of the data given showed that when wheat seedlings absorb nitrates, potassium must be available; the absence of any one of the other essential elements for a period of at least forty-eight hours for these young plants apparently was not injurious. Furthermore, seedlings which were exposed for twenty-four hours in a solution that contained equal concentrations of only magnesium and phosphate ions ($MgHPO_4$) sustained no apparent harmful effects (to either top or roots), but if they were placed for a similar length of time in equally dilute or diluter solutions of $MgSO_4$ or $Mg(NO_3)_2$, they sustained decided injury. $MgHPO_4$ seems to be a very good salt to supply the plants with magnesium and phosphate. Calcium-sulphate solution proved to be a medium which can be used effectively for young wheat seedlings for at least twenty-four hours and without any harmful effects. Furthermore, table 1 shows that when $MgHPO_4$ or KNO_3 was one of the salts of a type (see types II and III), larger plants were produced than when neither of these salts was contained in the type.

In recapitulating the conclusions, it seems that the availability and utilization of essential elements by wheat seedlings are not inconsiderably affected by the way in which these elements, presumably as ions, are paired. There undoubtedly is one kind of ion, or possibly more, of opposite charge with which any one of the essential ions can be used to best advantage by the plants. There is more than presumptive evidence in the results of this experiment that the proper pairing of nutrient elements, or of ions of opposite charge, is an important factor, and one that does account in a large measure for the physiological adaptability of a nutrient solution for plant growth. However, this pairing of any two ions in complete nutrient solutions does not mean that any definite cation-anion ratio must prevail. Investigations have shown that ionic ratios of nutrient solutions can cover a considerable range of values without any apparent physiological effect. However, the interpretation of this test should mean that if a nutrient solution is a poor medium for plant growth because of the large proportion of one ion, it should be improved by the addition of some other ion of opposite charge, even though this be added in the form of a salt that would also add more of the ion already in excess. That this is what actually happens, has been proven experimentally in this laboratory. The above conclusions may also be stated in other words, and, as a concrete example, $Mg(NO_3)_2$ can be used as the source of nitrates for plants in any growth media. But the utilization of nitrates by the plant will be largely influenced by the relative supply of available potassium in the medium; that is, the utilization by young wheat plants of the nitrate anion is closely related to that of the potassium cation, and *vice versa.* A cation-anion relation of other essential ions is apparent from a study of the results, but need not be considered in detail in this paper as the experiments will be discussed elsewhere. It may not, however, be amiss to state that the principle of the method here employed to study the physiological properties of solutions seems to give promise of yielding further information on the nutrition of the higher plants that can not be gained by the use of complete nutrient solutions because of their complexity.

University of California

VEGETATIVE VIGOR OF THE HOST AS A FACTOR INFLUENCING SUSCEPTIBILITY AND RESISTANCE TO CERTAIN RUST DISEASES OF THE HIGHER PLANTS

I

M. A. Raines

(Received for publication July 13, 1921)

Introduction

Studies on the cereal rusts were made covering various phases of the phenomena of rust epidemiology, including the effects of season, age of the host plant, etc., on its susceptibility and on the virulence of the disease; effects of varying dosage in securing inoculation; effects of the general nutritional condition of the host, etc. In carrying out these studies I have had the opportunity to convince myself of the frequently observed fact that health and vigor of the host favor rather than hinder its inoculation by a rust and the further development of the diseased condition. This observation has frequently been made and more or less casually reported in the literature on the rusts and other fungous diseases of plants. The significance of such observations in relation to general theories of immunity and resistance to disease has, however, nowhere been adequately recognized, and I have thought it worth while to bring together the available evidence bearing on this point.

It is a commonplace of pathological theory that the health and vigor of an organism and its susceptibility to disease are antithetic variables, that as one increases or is increased the other diminishes or is diminished correspondingly. Adami (1910,[1] 1: 409), summarizing the subject of predisposition to disease, lists the causes of acquired susceptibility as (1) social and environmental conditions; (2) injury; (3) malnutrition; (4) previous attack of the same disease or other infectious disease; and (5) exhaustion; all of them factors diminishing the vitality of the host. Zinnser (1914, p. 59), discussing the broader principles of infection and resistance, states:

> A person suffering from functional impairment of any kind is more likely to permit the invasion of a pathogenic microörganism than is a perfectly healthy well-nourished individual of the same species.

Kolmer (1917, p. 101) says, similarly:

> Acquired susceptibility . . . may be due to various factors, most of which lead to a state of reduced vitality, normal physiologic processes being impaired to a greater or less degree.

---

[1] The list of literature cited will be found at the conclusion of the second paper of this series.

Among plant pathologists, Jones (1905, p. 23) writes:

> Disease resistance and vegetative vigor are closely associated, although the factors involved are not necessarily identical. . . . So far as the evidence goes it seems to suggest that high vegetative vigor enables the plant to ward off in some degree the fungus attack.

In direct contrast with this view it must be noted that workers in the field of the rust diseases of higher plants have on several occasions been prompted to generalize to directly the opposite effect—that they found host vigor and susceptibility to disease not antithetic, and not independent, but parallel variables. Arthur (1903, p. 13), in a presidential address before the Botanical Society of America, stated from his long experience with plant rusts that

> So intimate is the association of parasite and host that as a rule the vigor of the parasite is directly proportional to the vigor of the host. Every culturist soon learns that to have success in his work he must employ strong, rapidly growing plants. Even if he succeeds in infecting weak plants, the fungus will rarely come to satisfactory fruitage.

Sheldon (1903, p. 74), summarizing his illuminating studies on the asparagus rust, concludes:

> Lowered vitality does not favor infection. . . . Whatever affects the growth of the asparagus has a like effect on the rust.

Stakman, in his extensive studies on the cereal rusts, on several occasions expresses similar views. Thus (1914, p. 40) he concludes that

> These experiments show that whatever is conducive to the vigorous development of the host is ordinarily conducive to the vigorous development of the parasite also.

And in another place (Stakman and Levine, 1919, pp. 75 and 76):

> Deficiency of soil moisture and sunlight and other ecological factors affecting the host plant unfavorably appear to be equally unfavorable to the rust parasite. . . . Adverse environmental conditions unfavorable to the host are also unfavorable for the parasite, affecting the virulence and spore size of the latter.

While the authors make their point incidentally and in no instance discuss their findings from the point of view of the general question of the relation between host vigor and susceptibility in infectious disease, it is evident that the question is suggested as to the possibly special and, from the point of view of the relation observed in the larger number of infectious diseases of plants and animals, peculiar relation between vegetative vigor of the host and susceptibility to infection in the rust diseases of the higher plants. The demonstration of such a peculiar relation would be of theoretical interest as limiting and qualifying the universality of the commonly accepted dictum that host vigor and virulence of disease are in inverse relation and would be of profound import in defining the practical problem of the prevention and control of the diseases concerned.

Review of Literature

Physiological studies on the rusts have been reported almost entirely from the point of view of a direct relation between the environmental factor concerned and the rust fungus, the essential intermediary between the two, the host, being kept more or less indistinctly in the background. A marshaling of the available data on the physiology of the rusts from the point of view of the present study, the possible correlation between host vigor and virulence of the parasite, reveals a very general agreement in favor of the concept that in the rust diseases of the higher plants there is a tendency for the parasite to exhibit a higher incidence of infection and greater virulence on the host of greater vegetative vigor. Similar instances in other classes of plant diseases are cited in the general discussion.

Host Nutrition: Nitrogen Nutrition and Mineral Nutrients

Field Studies

Butler (1918, p. 73) describes the coffee leaf disease (*Hemileia vastatrix* B. & Br.) as having first appeared on some of the best coffee in Ceylon, and states that it is, in the case of this disease, considered as established that fungous infection and growth occur better in strong leaves, rich in nutriment, than in those with less food supply.

The greater susceptibility to rust of wheat grown on highly fertile land has been noted repeatedly. Little (1883, p. 634) states that high manuring, especially with nitrogenous manures, predisposes wheat plants to rust. And Bolley (1889, p. 17) writes that

> It is a matter of common note that soils rich in organic plant foods, such as low-lying loams, are quite liable to produce rusted crops; and in England, where great quantities of nitrogenous fertilizers are used, much has been said as to the liability of the crop to rust upon fields to which such manures have been applied. Such observations go to confirm the belief that soils excessively rich in nitrogen, either natural or applied, produce wheat easily attacked by rust.

Freeman and Johnson (1911, p. 69), in their general review of the cereal rust problem in the United States, state:

> . . . It is now well established that where there is an excess of nitrogen in the soil, other things being equal, grains are more severely attacked by rust than crops on soil containing less nitrogen. . . . Where barnyard manures have been applied heavily the result is similar, and where grains are grown after a crop of clover, beans, or vetch, rusts may be expected. In fact it may be generally stated that where soils are rich in nitrogen, producing rank and succulent plant growth, rust attacks will, as a rule, be most severe on account of increased succulence of the plants, increased rankness of growth, delay in drying out after showers and dews, and slight delay in the ripening period. On the other hand phosphate of lime tends to shorten the ripening period and thus acts as a rust preventive to some extent. . . . In general, a rust attack is most virulent on a healthy plant.

Peacock (1911) states that rust in wheat and oats is favored by predisposition due to a too vigorous growth in early life. Biffen (1912), in his studies on the inheritance in wheat of rust resistance to *Puccinia glumarum*, found that the rust is most virulent when a complete fertilizer is used, and that the virulence of the disease decreases with a decrease in the amount of fertilizer. Comparing the two principal types of asparagus soils in California, the sediment and the peat, Smith (1905, p. 56) notes that asparagus growing in the latter soil is considerably more damaged by the same amount of disease. He comments that on peat formations, composed almost entirely of vegetable matter and water, a very luxuriant, quick-growing, tender, and succulent asparagus is produced.

Zavitz (1913) reports some very interesting observations on the relative susceptibility to rust of oats grown under conditions of varying thickness of seeding. The experiment was conducted through each of four years, using both large and small seed of heavy-stooling, medium-stooling, and light-stooling varieties of oats, and planting the seed of each variety in squares one, two, three, four, six, eight, and twelve inches apart. Table I is adapted from the data presented by Zavitz, and presents the average results of thirty-two tests made by planting oats at seven different distances apart. The results are the averages for four years.

Table I

Inches between Plants	Number of Heads per Plant	Height in Inches	Percentage Lodged	Days to Mature	Relative Yield per Plant		Percentage Rusted
					Straw	Grain	
1	1.0	20.4	5.6	91	100	100	11.8
2	1.1	27.8	11.9	93	361	457	15.0
3	1.3	32.6	12.8	94	782	1,227	17.8
4	2.0	33.1	29.9	95	1,179	2,031	20.9
6	4.2	35.3	35.8	97	2,823	4,402	25.4
8	6.5	34.9	34.7	99	4,389	6,645	27.7
12	11.2	34.9	30.1	100	8,475	10,320	33.2

The greater amount of rust observed with the increased distance between plants is best correlated with the increased luxuriance of growth exhibited by these plants. The difference of a week in the time of maturing between the most closely spaced and the most liberally spaced oat plants is hardly sufficient to account for the difference in the amount of rust infection. Observations were made at frequent intervals through the summer, and a rust difference due only to difference in time of maturity would not have shown up in this fashion in the data. A more logical explanation is the increase in the amount of lodging which closely parallels the increase in percentage of rust from the one-inch spacing to the six-inch spacing. But from the six-inch spacing to the twelve-inch spacing the amount of lodging decreases appreciably while the percentage of rust increases further, indicating that the increase in the amount of rust is independent of lodging. There is the strong suggestion, therefore, in Zavitz's data that the increase noted in the amount of rust present on oat plants grown at progressively greater distances apart is correlated with the increased luxuriance of growth of the host plants.

Water-Culture and Sand-Culture Studies

Ward (1902*a*) details two experiments on the susceptibility to rust infection of host plants which had been starved of essential nutrients. In the first experiment, 54 young seedlings of *Bromus secalinus* were used. The plants were grown in sand in 14 glass beakers, four to seven plants to a beaker, and watered with solutions of varying nutritive value. The plants in one beaker received only distilled water. The plants in another beaker received a cold-water extract of fresh horse dung, as a solution of high nutritive value. In a third beaker the plants received a full mineral nutritive solution (described as a "normal nutritive mineral solution containing nitrates, phosphates, and sulphates of potassium, calcium, and magnesium"). The remaining eleven beakers received an incomplete nutrient solution, the elements omitted being respectively K; N; Mg; Ca; P; Fe; N and Fe; Mg and Fe; Ca and Fe; P and Fe. Inoculation was effected by applying uredospores of *Puccinia dispersa* to the leaves by means of a swab of cotton; at the time of inoculation the seedlings were 16 days old, counting from the time of sowing.

Ward records detailed observations on the stature, robustness, color, and number of leaves of the seedlings in each beaker; on the time of appearance, number, and size of the pustules developed on them, and on the relative number of spores produced. Comparing the twelve seedlings which showed the most vigorous growth (6 which received the extract of horse dung, 3 the full nutrient solution, and 3 the full nutrient solution minus Fe, the plants averaging 20 cm. in height) with the ten poorest plants (4 receiving distilled water, 3 the full nutrient solution minus N, and 3 the nutrient solution minus N and Fe, the plants averaging 11 cm. in height), the observations recorded by Ward indicate that in the plants suffering from malnutrition (1) the incubation period of the rust was lengthened by one and two days; (2) the rust pustules were much smaller and produced fewer spores. In other words, a starved host meant a starved parasite. There is also the suggestion in the data that the starved seedlings showed a lower incidence of infection; but the small number of variables worked with, together with the large irregularity in dosage inherent in the method of inoculation used, compel reserve in making this deduction.

A second experiment with 64 seedlings, duplicating the first, gave similar results. Regarding the spores produced on well nourished and on starved seedlings, Ward states that microscopic examination revealed no differences. Spores from starved seedlings could produce infection on other seedlings, similarly starved.

In 1905, Ward reports experiments indicating that starving the host tissue after infection has taken place has an adverse effect on the growth of the fungous mycelium. He cut off infected leaves of cereals on the third day after artificial inoculation and floated them on water. Histological examinations of the leaves indicated that the rust fungus in the tissues continued to grow for a time, but soon showed signs of starvation.

Spinks (1913, p. 238) describes an experiment on the susceptibility to *Puccinia glumarum* of wheat plants grown in water cultures. He used six plants grown in each of three solutions: a standard nutrient solution (Detmer's); a nutrient solution containing four times the quantity of ammonium phosphate; and a nutrient solution containing four times the quantity of potassium chloride. The cultures were inoculated by applying uredospores to the leaves; they were then set outdoors, so that further spread of the rust occurred naturally. Spinks gives no data on the condition of the plants, and this can only be inferred from the mode of treatment they received. The data presented indicate that the plants growing in the nutrient solutions containing a four-fold concentration of nitrogen were more susceptible than those in the standard solution. Excess concentration of KCl gave an apparent slight depression of susceptibility.

Stakman (1914, p. 39) reports some experiments with *Puccinia graminis tritici* on wheat seedlings grown in water cultures. In an experiment in which nitrogen and phosphorus were omitted from the culture solutions, the check plants were more severely attacked than the experimental plants. Summarizing his results, Stakman says (p. 48):

> It was found that in general the absence or presence in excessive amounts of various nutrient substances, such as nitrogen and phosphorus salts, did not directly affect the immunity or susceptibility of wheats. Conditions favoring a normal development of the host were conducive to a vigorous development of the rust. The action of fertilizers, either natural or artificial, is probably indirect.

Soil-Culture Experiments

Sheldon (1905, p. 226) remarks on the low susceptibility shown by poorly growing carnations to artificial infection with *Puccinia Caryophylli:*

> The results show that the plants that were making a vigorous growth were more susceptible to artificial infection than those that were making little or no apparent growth. A few slowly growing plants were repeatedly inoculated without success until the plants were given extra care and stimulated so that they began to grow more vigorously. Some carnations, grown in small pots, were each inoculated five or six times at intervals of about twenty days without any of the inoculations being effective. These plants grew very slowly, were slender, and produced only one, or at most two, small blossoms.

In the same paper (p. 228) Sheldon reports an experiment on the length of the incubation period of the carnation rust in which he inoculated simultaneously 170 pinks growing in soils of varying nutritive values. The plants had been derived by taking sets of cuttings from the same stock plant, a green-leaved pink known to be very susceptible to carnation rust, and they were grown in five different soils ranging in composition from one that was principally sand to one containing chiefly organic matter. Sheldon's observations indicated that the growth of the host varied directly with the amount of organic matter, nitrogen, and silt in the different soils; and that with increased vigor and growth of the host the incubation period of the fungus decreased in length, from 21 days in the poorest plants to 16 days in the most vigorous individuals.

Miss Gibson (1904, p. 188) describes an experiment on the effects of nutritional treatment on the susceptibility of certain varieties of chrysanthemum to rust.

> The young plants were then divided into four groups. *A* were fed as if for exhibition; *B* were grown normally; *C* were starved by being grown in small pots; and *D* were grown in a warm greenhouse.

The rust developed on all the plants, but a luxuriant state of growth of the host favored the greater development of the fungous mycelium.

Spinks (1913, p. 240) conducted an experiment on the susceptibility to rust of wheat plants grown in pots receiving different nutritive solutions. The data indicate somewhat higher susceptibility on the part of the plants richly fed with nitrogen. Stakman (1914, p. 16) finds that high fertilization of the soil increased the susceptibility to infection by *Puccinia graminis tritici* of resistant varieties of wheat, and so concludes that high fertilization is conducive to increased severity of rust attack on very resistant varieties as well as on susceptible forms.

Indicating that the effect of specific nutrient substances is indirect, secondary to their effect on the vigor of the host, is the observation by Stakman and Levine (1919, p. 72) that an application of sodium nitrate, excessive to the point of inhibiting the growth of the host, also inhibits the development of the rust and diminishes very perceptibly the size of the urediniospores. Ward (1902*a*) states that the size of the uredospores was not affected by starving the host. Stakman and Levine arrived at their observation that the size of the spores is affected by biometrical methods, which would cause to stand out distinctly size differences not apparent on gross examination. Ward does not give any spore measurements.

Host Nutrition: Carbon Metabolism

Intimate relation between the progress of a rust infection and the carbon metabolism of the host tissue has been demonstrated by Ward (1905), Fromme (1913), and Mains (1917). Sheldon (1903) studied the effect of light and temperature on rust development, and Stakman and his co-workers (1917, 1919) have studied quantitatively the effects of light on the cereal rusts.

Ward (1905, p. 40) refers to experiments on cereal rusts indicating that when, shortly after inoculation, the host is placed under conditions where it cannot manufacture carbohydrates, as by keeping it in the dark or in light from which the red-orange end of the spectrum is filtered off or in air deprived of carbon dioxide, the development of the rust is inhibited.

Fromme (1913, p. 516) found that placing oat plants recently inoculated with *Puccinia coronifera* in the dark for a period of several days increased the length of the incubation period of the rust by a corresponding interval. Fromme interprets the observation to indicate that the fungus is dependent for its nutrition on some intermediate product of photosynthesis.

Mains (1917, p. 191) confirms Fromme's observation that the development of the crown rust of oats is retarded in the absence of light, and adds that if the infected plant is left in the dark too long (which would greatly impair the vitality and vigor of the host tissue) the rust is killed. Mains also independently repeated Ward's observation that growing the host plants in an atmosphere free from carbon dioxide inhibits the development of the rust. Similar experiments with *Puccinia Sorghi* on seedling plants of *Zea Mays*, however, failed to arrest the development of the rust. Further experiments showed that if the host leaf is supplied with carbohydrates, either from the reserve stores of the endosperm or by being floated under aseptic conditions on a sugar solution, then the rust develops successfully, even if, because of the absence of light or of carbon dioxide, the host tissue cannot manufacture its own carbohydrates. Mains is therefore prompted to qualify Fromme's inference that the rust is dependent upon intermediate products of photosynthesis into the statement that the rust is dependent upon transitory carbohydrates.

It does not necessarily follow from an observation that the development of the rust is inhibited upon a plant starved of an essential nutrient—whether carbon, or nitrogen, or potassium, etc.—that the rust fungus is dependent for its nutrition upon compounds of that substance. As long as the host plant is at all alive, or, even if it is dead, before disintegration of its substance has set in, it contains carbon, nitrogen, etc., compounds, and we cannot say that the rust could not develop because of the absence of such compounds. Such observations are best interpreted on the basis of the physiological condition of the host when it is starved of an essential nutrient substance. We can not say that a host plant starved of an essential nutrient is a host plant deficient in that particular class of substances; but we can say that a host plant starved of an essential nutrient is a host plant that is not assimilating, that is not growing, a plant in which anabolic processes are at a standstill and katabolic processes predominate. And we are justified in inferring from the observed behavior of rust fungi on host plants starved of essential nutrients that a plant which is not assimilating, which is not growing, in which anabolic processes are at a standstill and katabolic processes predominate, does not make a congenial host for the rust fungus. The suggestion that the rust is dependent for nutrition upon some particular class of substances within the host is strongest in the case of the carbon compounds, because of the relatively large amounts of carbon needed by the growing plant and because of the facility and exactness with which the growth and vitality of the host plant can be experimentally controlled through this phase of its metabolism.

Stakman and his co-workers have studied the light relations of *Puccinia graminis tritici* inoculated on seedling plants of wheat. Stakman and Piemeisel (1917, p. 487) state that a considerable amount of sunlight is necessary for the best development of the rust. They found that during periods of cloudy weather the incubation period may be lengthened a week or more, and that the rust does not develop so abundantly as during bright weather. Shaded plants invariably were more weakly infected than the others. Partially etiolated plants were infected with difficulty, and the rust developed very weakly on them. No rust developed on etiolated plants. Stakman and Levine (1919, p. 71) found that the rust developed considerably better in fairly high intensities of light than under conditions of less favorable illumination. The size of the urediniospores responded in a similar manner. They summarize their observations on the light relations of the rust as indicating that

> . . . In as much as the photosynthetic activities of the host plant are affected by the light intensity, in so much does the structure and function of the rust depend on the same factor.

Water Relations

While many observations have been made on the relation of moisture conditions of soil and air to rust virulence on plants in the field, they are subject to criticism in that they do not distinguish between the effect of the moisture conditions on uredospore germination and penetration, and the effect on the vigor of the host and the progress of the rust infection in its tissues. Abundant moisture is always favorable to uredospore germination and infection, but the effect on the physiology of the host is specific for the plant. Abundance of moisture will favor the growth of a mesophyte, but it will have a depressing effect on the vigor of a plant of xerophytic tendencies. Limiting ourselves, therefore, to observations when inoculation was artificially effected under conditions of maximum atmospheric humidity, the evidence permits the inference that the moisture conditions of atmosphere and soil most favorable for the growth of the host plant are likewise optimum for the growth and sporulation of the rust.

The most complete and suggestive experiment is reported by Stakman (1914, p. 35). Wheat plants of both drought-resisting varieties and ordinary mesophytic types were employed, and they were grown in two series. The soil in one series was kept very wet, while that in the other series was kept as dry as possible without endangering the life of the plants. On inoculation with rust, the drought-resisting forms exhibited better infection in the dry soil, while the mesophytic types showed slightly greater virulence of disease in the moist soils. Repeated trials were made with substantially the same result. Stakman concludes:

> It is probable then that, conditions having been favorable for a rust infection, the water relation in the soil which is most favorable for the host plant's development is also the most favorable for the development of the rust.

Mains (1917, p. 189) found that the development of *P. Sorghi* on corn, as shown by the number of pustules produced, is favored by a humid atmosphere and by a wet soil—conditions favorable to the growth of the corn plant. The length of the incubation period was not appreciably influenced.

Stakman and Levine (1919, p. 45), in experiments to determine the length of time that wheat seedlings inoculated with *P. graminis tritici* should be kept in a saturated atmosphere in order to obtain maximum infection, found that keeping the plants under a bell jar for more than 48 hours reduced the amount of infection obtained and appreciably lengthened the incubation period. In other experiments (p. 70) they noted a tendency for excessively high or excessively low humidity during the incubation period to cause a decrease in the size of the urediniospores. In another experiment on soil moisture (p. 71) in which three series of plants were employed, one of which was heavily watered, the second moderately, and the third received only enough water to prevent the plants from wilting, Stakman and Levine found that the plants in the wet soil were more severely attacked and that the urediniospores developed on them were larger than those in the other two series. The plants that suffered from drought produced the smallest spores. The authors conclude as a result of their study on the effect of environmental factors on the morphology of the urediniospore of *Puccinia graminis tritici* that deficiency of soil moisture and of sunlight and other ecological factors affecting the host plant unfavorably appear to be equally unfavorable to the rust parasite.

Temperature Relations

There is abundant evidence of a tendency towards physiological parallelism of host and rust in their temperature relations.

Sheldon (1903, p. 33) studied the relation between greenhouse temperature and hours of sunshine per day, and the length of the incubation period in the asparagus rust. His experiments extended over a period of five months, from December, 1900, to May, 1901, and yielded data on 132 asparagus plants. The results indicate an inverse relation between the temperature and light conditions under which the host was growing and the incubation period of the rust. During December and January the length of the incubation period was regularly 14 to 17 days. During April and May, when the day was longer, the light better, and the temperature higher, the length of the incubation period was only 8 to 10 days.

Similar experiments with the carnation rust (*Puccinia Caryophylli* on *Dianthus sinensis*) gave opposite results. The incubation period increased, from 15 days in January to 21 days in May. In explanation, Sheldon suggests the possibility that the temperature and light in the greenhouse were better suited to the asparagus than to the pinks. In Bailey's *Cyclopedia of Horticulture* (1914, p. 670) the carnation is characterized as a cool-temperature plant.

Several observers comment on the lengthening of the incubation period in cold weather, and the difficulty of obtaining infection in very warm weather. Christman (1905, p. 106) found that in the cooler weather of spring in Wisconsin the incubation period of cereal rusts is usually lengthened to between three and four weeks. Ward (1902*b*, p. 233) remarks that, in working with the brown rust of the bromes, he found infection difficult to carry out in hot weather; and in a succeeding paper (1905, p. 41) he repeats and emphasizes the significance of the observation. In this paper (p. 39), Ward also refers to experiments in which the normal development of the rust was interfered with by warming and chilling the root system of the host plant. Butler and Hayman (1906) describe unsuccessful efforts to produce artificial rust infection on plants growing in the open in the hot weather in India, and express doubts whether the uredospores have power to infect when exposed to temperatures exceeding 100° F. Fromme (1913) found that temperatures below 20° C. increased the incubation period of *Puccinia coronifera* on oats.

Stakman (1914, p. 30), in his culture work with cereal rusts, observed the incubation period to vary with temperature conditions, both high and low temperatures lengthening the period very perceptibly. Mains (1917, p. 187) observed that low temperatures retarded the development of *P. coronata* and *P. Sorghi* in the host. Stakman and Levine (1919, p. 68) report the optimum temperature for *P. graminis tritici* to be between 66.5° and 70° F., this giving the shortest incubation period, the most vigorous infection, and the largest urediniospores, for the host employed. At a higher temperature than 70° F. the development of the uredinia was retarded at the rate of one day for every 10 degrees' rise in temperature, but rust developed at as high a temperature as the host endured although the size of the urediniospores produced was considerably decreased. At low temperatures the development of the uredinia was retarded at the rate of one day for every 5 degrees' fall in temperature. Infection resulted at as low temperatures as the host could stand. The spores at the lower temperatures were rather small, but the difference was not as great as in the case of the high temperatures, with moderate temperatures as the basis for comparison.

Lauritzen (1919, p. 19) reports experiments indicating that 42° F. is below the minimal temperature at which *P. graminis tritici* is able to infect wheat. Above this temperature the amount of infection rises rapidly until at 53° F. it approaches the average for the higher temperatures. The highest temperature at which the rust will produce infection in wheat was found to be 80° F. under the conditions of the experiments. The figures obtained by Johnson (1912) are cited in evidence that it is not failure of the spores to germinate which determines the infection limits observed in the experiments. Johnson (p. 48) found the optimum temperatures for the germination of the uredospores of the common cereal grain rusts to be low —12° to 17° C.—helping to explain such observations as the difficulty of keeping rust in culture in the greenhouse in the summer, when the incubation period of the rust is shorter than at any other time of the year but it is remarkably difficult to obtain infection; the difficulty of finding viable uredospore material in the spring, the larger number of the spores having already germinated; and the favoring of rust development and epidemics by subnormal temperatures at the critical infection periods in the life of the host plants.

Stimulants and Depressants; Toxic Agents

Observations on chemical and physical agents stimulating or depressing the vitality of the host plant indicate that susceptibility to rust infection is affected in like manner.

Sheldon (1903, p. 44) found that in the case of the asparagus rust and the carnation rust complete immunity to infection can be produced by lowering the vitality of the host—an end the failure to achieve which in the case of the brown rust of the bromes caused disappointment to Ward. Concerning the asparagus rust, Sheldon states (p. 44):

> Attempts have been made repeatedly not only on asparagus but on several species of pinks, to inoculate them when they are not growing well. It was tried on repotted plants, those attacked by insects, and young seedlings. A failure was the result in nearly every case; while with vigorous, growing plants which had become established there were few failures—thirty-seven out of forty-two inoculations made at one time being successful in one instance—approximately 90 percent.

In his experiments with the carnation rust Sheldon (p. 83) found attacks by thrips a very disturbing factor, as it was almost an impossibility to secure infection where the thrips had worked to any extent either before or after inoculation, while vigorously growing plants which were free from thrips were readily inoculated.

Spinks (1913, p. 243) and Voelcker (1912, p. 319) have made observations on the susceptibility to rust of the wheat plants grown in the pot-culture experiments on the fertilizing effects of small quantities of the salts of the heavy metals conducted at Woburn, England. They found that the lithium salts of the 1911 experiments depressed susceptibility, with the exception of lithium nitrate which gave increased susceptibility to rust. The experiments of 1912 were with zinc salts, and they were all found to produce increased susceptibility to rust, zinc nitrate seeming particularly notable in this respect.

Stakman (1914, p. 15) increased the susceptibility of cereals to rusts to which they are ordinarily highly resistant by slight anaesthetization with ether and chloroform. Jost (1907, p. 195) states that weak etherization accelerates respiration, and such treatment is usually considered as stimulating the metabolism of the plant. Stakman (1914, p. 39) also describes an experiment in which it was sought to influence the susceptibility of wheat plants grown in water cultures by introducing various salts into the culture solution. Copper sulphate, copper carbonate, and iron sulphate were added in varying amounts. The results showed that none of the salts experimented with appreciably decreased the amount of rust when used in such concentration as to permit the normal development of the host plant. Infection was secured on all the plants, even those which were stunted to one sixth their normal size.

Eriksson and Hammarlund (1914) report partial success in delaying and inhibiting the development of *Puccinia malvacearum* on *Althea rosea* by treating the soil with a 3 to 5 percent solution of copper sulphate. They give no data on the vigor of the plants.

Bailey (1920, p. 76) found that hollyhock plants stunted by red spider showed comparative immunity to rust.

Trauma

There are only two recorded experiments on the effect of trauma on susceptibility to rust; they do not agree in their findings. Trauma usually has an immediately stimulating effect on the metabolism of a plant tissue and might be expected to increase susceptibility to rust. Hecke (1915) mentions that Barfuss working in his laboratory has demonstrated that wheat rust, which ordinarily does not go to rye or barley, readily infects rye and barley leaves if they have previously been injured. After cultivating the rust for seven generations on wounded leaves, Barfuss succeeded in definitely obtaining infections on uninjured leaves; but these did not mature spores. The rust did not lose its power to go back to wheat. This is much after the manner of Salmon's work in increasing the susceptibility of resistant host plants to Erysiphaceous parasites.

Similar efforts to these by Stakman, also working with cereal rusts, gave negative results. Stakman (1914, p. 16) found leaf injury to have no effect on susceptibility to rust.

In one experiment 16 leaves were pricked full of holes in an area of one centimeter or more. They were then inoculated and 4 became flecked, but no pustules developed. Histological examination showed that the spores had sent out germ tubes in large numbers. These grew among the host cells, but true infection did not take place. Sections of these plants were made and examined. It was clearly evident that leaf injury did not increase the chances for infection.

Age and Maturity of Host Tissue

In his experiments with the asparagus rust Sheldon (1903, p. 47) found a great difference in susceptibility in favor of young and vigorously growing shoots as against older shoots of the same plant. His observations indicated that

> The incubation period of the rust on plants of the same age and growing in the same pot so that conditions were practically identical, was very uniform. When the plants were of the same age and growing in the same kind of soil in different pots, there was still a uniformity. But when there was a difference in maturity, as of two shoots from the same root, or several plants growing in the same pot, there was a difference in the time—of four days in one instance, the young growing shoots showing sori first. The sori showed first on the young growing shoots, and developed faster and to larger size. The more robust the shoot, the larger the sori were and the more spores they produced.

Sheldon found practically no difference between young shoots of young and old plants. A few shoots from three-year-old plants growing in the same pots showed rust the same day that the seedlings did. Sheldon made similar observations on the carnation rust.

An age factor of a different kind is reported by Sheldon (1905, p. 227) in the susceptibility of onions to *Puccinia Asparagi*. Complete immunity was found when seedling onions were inoculated with the rust. The inoculations were begun as soon as the seedlings appeared above ground, and were repeated at intervals until the seedlings were two months old, when almost every inoculation was successful.

Galloway (1903, p. 208) reports a maturity factor as seemingly affecting the susceptibility of wheat to rust. In his extensive experiments on the possibility of controlling cereal rusts by means of spraying or soil treatment, Galloway found that the rust, while abundant on the primary experimental plots, was absent from nearly all the duplicate plots. The latter had been planted a week to ten days later than the original plots, and in point of growth were at least as much behind them at the time of observation. As the experiments were with a winter wheat, planted the preceding fall, it cannot have been that the plants of the duplicate plots had missed a wave of inoculating material.

Miss Gibson (1904, p. 188) reports the presence of a seasonal factor in the susceptibility of certain varieties of chrysanthemum to the chrysanthemum rust. She noticed that certain varieties do not take the rust in summer, although growing in the midst of plants thickly covered with it. As the rust spores germinate well in summer and the germ tubes penetrate readily, it is a problem not in the physiology of the parasite but rather in that of the host.

Stakman and Piemeisel (1917, p. 486), in their extensive inoculation work with cereal and grass rusts, found the cereals and *Dactylis glomerata* apparently equally susceptible at all ages up to ripening time. Agropyron and Elymus were extremely susceptible when young and much less so when older. On the other hand, *Phleum pratense* and *Agrostis alba* were more susceptible when older.

Stakman and Levine (1919, p. 73) observed an age difference in the progress of an infection of *P. graminis avenae* on oats. Plants that were one week old at the time of inoculation were somewhat more heavily infected at first than plants one, two, and four weeks older; but at the end of ten days the infection was heavier on the older plants, especially so on the plants that were three weeks old at the time of inoculation. The size of the urediniospores was uniform regardless of the age of the host, nor was any difference observed in the shape and color of the spores. They also state that

> . . . the junior author has obtained very successful infection on mature plants of more than a hundred different varieties of wheat, grown in the greenhouse and artificially inoculated with *P. graminis tritici.*

Giddings (1918, p. 33) found susceptibility in apple leaves to infection by *Gymnosporangium juniperi-virginiana* to be limited to young leaves, not more than fifteen to twenty-four days old after unrolling from the bud, older leaves being almost completely resistant. H. H. York (personal communication) has found that the very young leaves of Ribes are resistant to infection by *Cronartium ribicola,* susceptibility not appearing until some time after the unfolding of the leaf.

The factor of age and maturity of host tissue is reviewed here briefly because of its close association in thought with vegetative vigor. There is no necessary physiological connection between the two factors, and their significance in susceptibility and resistance to rust infection is probably of a different nature. The age factor in disease resistance is probably to be classed rather with varietal and constitutional differences than with physiological condition.

Field Studies and Experiments

A series of studies was made to determine the general facts as to the occurrence and epidemiology of the rusts on the cereal grains in the so called local-flora region of New York, and especially for the New York Botanical Garden and vicinity. Data as to the points involved were found to be very meager, and these preliminary studies were made as a contribution to the general problem of rust epidemiology in the Atlantic States, a field so far little studied because of the relative unimportance of grain growing in these regions. More especially also it was desired to lay a foundation for future studies of rust problems which presuppose a knowledge of the general behavior of the rusts under the climatic and other conditions of the region.

The Rusts of the Cereal Grains and Related Grasses in the Vicinity of New York

The time of first appearance, period of greatest virulence, and even the identity of the rusts on the cereal grains and related grasses which have been suspected of harboring cereal rusts in the vicinity of New York City cannot be regarded as accurately determined.

The herbarium of the New York Botanical Garden contains 17 collections of four different species of rusts on cereal grains and grasses which might bear related rusts from the region about New York City, the so called local-flora region, as follows:

Puccinia rubigo-vera DC. (*Puccinia dispersa* Eriks. & Henn. *ex parte*):
  On wheat, at Cedarville, N. J., June 3, 1880. Ellis collection (II).
  On rye, at Flatbush, L. I., May 25, 1889, by Zabriskie (II).
Puccinia poculiformis (Jacq.) Kuntz (*Puccinia graminis* Pers.):
  On wheat, at Greencastle, Putnam Co., N. Y., September, 1893, by L. M. Underwood (II, III).
  On oats, at Greencastle, Putnam Co., N. Y., September, 1893, by L. M. Underwood (II, III).
  On oats, at the New York Botanical Garden, October, 1900, by the class in mycology (II, III).
  On *Anthoxanthum odoratum*, at Newfield, N. J., May 4, 1890. Ellis collection (III).
  On *Ammophila brevipile* Torr., at Egg Harbor, N. J., 188–, by S. M. Tracy (III).
  On *Agrostis vulgaris* L., at Plainville, Conn., August 23, 1883, by A. B. Seymour (II, III).
  On *Poa pratensis* L., at Greencastle, Putnam Co., N. Y., September, 1893, by L. M. Underwood (III).
  On *Berberis vulgaris* L., at Newfield, N. J., May 24, 1875. Ellis collection (I).
  On *B. vulgaris*, at Newfield, N. J., May, 1881. Ellis collection (I).
  On *B. vulgaris*, at Richmond Hill, L. I., May 22, 1889, by S. E. Jelliffe (I).
Puccinia andropogonis Schw.:
  On *Aureolaria villosa* (Muhl.) Raf. (*Gerardia villosa* Muhl.), at Newfield, N. J., June, 1874, by Gerard (I).
  On *Gerardia quercifolia* Pursh., at Westville, Conn., June, 1890, by R. Thaxter (I).
Puccinia Ellisiana Thum.:
  On *Andropogon* sp., at Newfield, N. J., September, 1897. Ellis collection (III).
  On *Andropogon Scoparius* Michx., at New Haven, Conn., October 4, 1913, by J. M. Bates (III).
  On *Viola pedata* L., at New Haven, Conn., May 31, 1841, by Manlius Smith (I).

I have also found the following notes on the occurrence of these forms in the local-flora region. Peck (1871, p. 121), in his list of the Pucciniae of New York State, lists *P. coronata* Cord. as common on the leaves of grasses and cereals in August and September, and *P. graminis* Pers. as common on the leaves and sheaths of grasses and cereals in autumn and spring. Thaxter (1890, p. 98) notes the abundant occurrence of *P. rubigo-vera* DC. (probably *P. dispersa* Eriks. and Henn.) on rye in Connecticut in the year 1890, describing it as covering the leaves with its rust-covered uredo form and doing considerable damage.

Humphrey (1891, p. 228) remarks on the occurrence of rust (*Puccinia* sp.) on rye in Massachusetts in 1891. He records some interesting observations on the mode of wintering over of the fungus. The rust, he notes, appeared on rye in June so abundantly that the spores rose in clouds when the plants were touched. In July this stage of the fungus had largely disappeared, and the winter pustules were mainly in evidence. Observations seemed to indicate that the rust does not survive the winter in its host plant but depends upon fresh infection in the spring on the plots of the Massachusetts Agricultural Experiment Station. In this connection Humphrey notes that uredosori on rye seedlings survived the early frosts and seemed vigorous until the heavy frosts and snowfalls. The plots were then covered with snow until spring. When they were again exposed, the discolored spots where the pustules had been could be readily observed, and examination showed mycelium to be present in the spots; but it was apparently dead, for repeated examination of the plot failed to detect new spores breaking out from any of the old spots. The fungus was not observed after growth was resumed until early in June.

Jelliffe (1889, p. 35) reports *Puccinia graminis* Pers. on the barberry and on wheat as of frequent occurrence throughout Long Island. Clinton (1903) reports the occurrence in Connecticut of *P. rubigo-vera* DC. (probably *P. dispersa* Eriks. & Henn.) on rye and barley, of *P. graminis* Pers. on rye, barley, oats, red-top, and timothy, and of *P. coronata* Cord. on the leaves of *Rhamnus cathartica*, *Notoholcus lanatus*, and *Avena sativa*. The outbreaks of the last, Clinton notes, are not nearly so prolonged or prominent as those of *P. graminis*. Burnham and Latham (1914) report finding *P. coronata* Cord. on the leaves of *Rhamnus cathartica*, *Notoholcus lanatus*, and *Avena sativa* at Sothold, L. I. They also report the occurrence there of *P. graminis* Pers. on *Berberis vulgaris* and on the leaves of various grasses, and of *P. triticina* Eriks. on various species of Triticum.

During the summer and fall of 1916 the writer collected the following cereal and grass rusts in the so called "local-flora region," *i.e.*, the region within one hundred miles of New York.

Puccinia dispersa Eriks. & Henn.:

On wheat, five collections: at Lakehurst, N. J., June 6 (II) and August 23 (II, III); at Yonkers, N. Y., July 16 (II); at New Brunswick, N. J., July 24 (II); at Williamsbridge, N. Y., July 19 to September (II, III); at the New York Botanical Garden, July 17 to October (II, III).

On rye, eight collections: at Tom's River, N. J., June 3 (II, III); at Lakehurst, N. J., June 20 (II, III); at Yonkers, N. Y., July 16 (II); at Queens, L. I., June 15 (II); at New Brunswick, N. J., July 24 (II); at Williamsbridge, N. Y., July 19 (II); at the New York Botanical Garden, July 17 to October; at Nyack, N. Y., July 8 (II).

On barley, two collections: at Williamsbridge, N. Y., September 13 (II); at the New York Botanical Garden, September 15 (II).

On *Agropyron repens*, three collections: at Tom's River, N. J., July 4 (II); at Williamsbridge, N. Y., July 19 (II); at the New York Botanical Garden, July 8 to October (II, III).

These rusts correspond closely with Eriksson and Henning's *Puccinia dispersa* (*Puccinia rubigo-vera* DC. *pro parte*). The uredo of the above-listed collections is amphigenous. The number of pustules on the upper surface is somewhat in excess of that on the lower surface, counts on pieces of leaf 1 cm. in length giving the numbers 174 to 158, and 217 to 195 respectively. The sori on the upper surface are more pulverulent.

The uredos on the wheat, rye, and Agropyron were certainly not distinguishable by morphological characters. The pustules on the barley show very little pulverulence. Measurements of the pustules on the barley (measurements being made on the size of the rupture in the epidermis) show them to be of the same size as the pustules on the other hosts of *P. dispersa*. The spores of the barley rust are narrower than those on the other cereals. Twenty-five spores gave an average ratio of length to width of 1.218. Seven such measurements on wheat gave ratios ranging from 1.083 to 1.163.

Puccinia graminis Pers.:

On *Phleum pratense*, at Tom's River, N. J., August 23. Only II was found on this host, as others have noted.

On oats, at Tom's River, N. J., August 23 (II, III); at Williamsbridge, N. Y., August 16 (II, III); at the New York Botanical Garden, August 26 (II, III). The rust was never abundant, occurring as a thin sprinkling on the leaves and sheaths among the crown-rust sori, and on the culms. The long, rectangular, erumpent teleutosori are very conspicuous scattered among the mass of smaller, covered sori of the crown rust.

Puccinia coronifera Kleb.:

On oats, at Williamsbridge, N. Y., August 2 to September (II, III); at the New York Botanical Garden, August 12 to October (II, III); at Tom's River, N. J., August 23 (II, III).

The crown rust of the present collections differs in some minor particulars from the published descriptions I found it somewhat later in its appearance on the sheath, but both uredo and teleuto in the end are as abundant on the sheath as on the leaf blade. Eriksson and Henning (p. 240) found it "rarely on the sheath." Both the uredo and the teleuto occur in abundance on the lower and the upper surfaces of the leaves, but the uredo is always in excess on the upper surface while the teleuto first appears and is always in advance in its development on the under surface. Grove (p. 256) and Fischer (p. 375) describe the teleuto as hypophyllous. The most considerable discrepancy between the descriptions of Grove and Plowright and the rust here described is in the number of the germ pores. Five spores of which the germ pores were counted showed respectively 9, 9, 9, 11, and 8 pores. The germ pores as well as the spines are inconspicuous until brought out by treatment with lactic acid. Grove (p. 256) and Plowright (p. 164) describe the germ pores as 3 to 4 in number. Fischer (p. 275) simply refers to them as inconspicuous, and Eriksson and Henning (p. 240) do not mention them in their description of the crown rust.

Puccinia impatientis (Schw.) Arth.:

On *Elymus virginicus* L., at Valley Stream, L. I., August 12 (II); at Hackensack, N. J., September 18 (III); at the New York Botanical Garden, June to October (II, III).

At the last-named station *Impatiens aurea* Muhl. near by was infected with an aecidium. The uredospores of this form are markedly less rounded and the teleutosori are much longer than in the case of the wheat and rye rusts as I have found them. The rust on Elymus has, however, been classed with *P. rubigo-vera* DC.

Puccinia poculiformis:
On *Cinna arundinacea*, at Jerome Avenue, New York City, September 15 (III).

An Experiment to Determine the Time and Method of First Appearance of the Rust, in which the Source and Condition of the Seed as Possible Factors are also Tested

In investigations of a physiological nature on the cereal rusts it is desirable that the probability be established that, for the variables being compared, the source of the fungus being worked with is constant. This point in experimental method assumes particular importance, in investigations such as are the subject of the present paper, from the recent demonstration of the variation in physiological properties of strains of rust from different localities. Assuming that the rust endemic in any locality is fairly constant in its behavior (whether the constancy is due to actual genetic purity, or to an admixture of strains in constant proportions), a possible source of error would be the seed transmission of the rust, making the source and condition of the seed a factor in determining the nature of the rust. Critical evidence on the question of the rôle played by the seed in the first appearance of rust on cereals in early summer is still lacking. As against the feeling of necessity, almost, in the minds of some investigators, of the assumption of seed transmission of the rusts in explaining certain phenomena in the epidemiology of these diseases, must be counted the inability to demonstrate with certainty a means of transmission, or to observe seed transmission of cereal rusts under controlled experimental conditions.

To test the possible rôle played by the seed in determining the first appearance of rust under field conditions in the vicinity of New York, 25 patches of wheat, 8 patches of barley, one patch of rye, and one patch of oats were planted with seed from widely different sources, and of varying age and condition on a plot of ground near the New York Botanical Garden. The plantings were made on high, well drained land which had not been cultivated since 1912, when it had been put to corn. The nearest plot of cereal was a field of oats about one quarter mile away, and there were no other grain fields within a distance of one mile at least. It is not thought that this single experiment is of very great significance as to the general question of possible seed transmission of rust. It is of interest, however, to include such inferior seed as that planted in the first three plots in a test as to the time of appearance of the rust.

The varieties planted, the source of the seed, and the date the rust first appeared on the plants are shown in table 2.

TABLE 2

(The designations "screening wheat," "smutted wheat," etc., are the millers' terms)

Plot No.	Cereal Planted	Variety	Source of Seed	Date Rust First Appeared
1....	Wheat	"Rusted wheat"	Fargo Milling Co., Fargo, N. D., 1915	July 19
2....	“	"Smutted wheat"	Washburn-Crosby Co., Minneapolis, Minn., 1915	“
3....	“	"Screening wheat"	Fargo Milling Co., Fargo, N. D., 1915	“
4....	“	Haynes Bluestem	Arlington Station, 1912	“
5....	“	Minnesota no. 163	Akron Substation, 1912	“
6....	“	“ no. 163	Arlington, 1912	“
7....	“	“ no. 188	Minn. Exp. Station, 1912	“
8....	“	“ no. 181	Minn. Exp. Station, 1912	“
9....	“	“ no. 169	Minn. Exp. Station, 1912	“
10....	“	Rural New Yorker	Arlington Station, 1912	“
11....	“	Early Genesee Giant	Akron Substation, 1912	“
12....	“	Dawson's Golden Chaff	Arlington Station, 1912	“
13....	“	Early Red Chief	Arlington Station, 1912	“
14....	“	Seneca Chief	Wis. Exp. Station, 1912	“
15....	“	Fish-head	Minn. Exp. Station, 1912	“
16....	“	Red Russian	Akron Substation, 1912	“
17....	“	Amantka	Akron Substation, 1912	“
18....	“	Galgalos	Akron Substation, 1914	“
19....	“	Little Club	Arlington Station, 1913	“
20....	“	Wasnani	Akron Substation, 1913	“
21....	“	Erivan	Akron Substation, 1913	“
22....	“	Ghanooka	Wis. Exp. Station, 1912	“
23....	“	Beloturka	Akron Substation, 1912	“
24....	“	Macaroni	Wis. Exp. Station, 1914	“
25....	“	Rupert's Giant	Thorburn Co., 1916	“
26....	Rye	Spring	Thorburn Co., 1916	“
27....	Oats	Tartar King	Thorburn Co., 1916	August 2
28....	Barley	White Smyrna	Akron Substation, 1912	Sept. 13
29....	“	Chevalier	Minn. Exp. Station, 1912	“
30....	“	Black Hulless	Hays Exp. Station, 1913	“
31....	“	White Hulless	Hays Exp. Station, 1913	“
32....	“	Swankali	Minn. Exp. Station, 1912	“
33....	“	Telli	Arlington Station, 1912	“
34....	“	Gaitami	Arlington Station, 1912	“
35....	“	Wis. Ped. no. 5	Minn. Exp. Station, 1913	“

As the table shows, the rust appeared simultaneously on all the plots of wheat and barley respectively. It appeared at the same time on a resistant variety such as the Macaroni wheat and on a susceptible variety such as Rupert's Giant; on plants grown from seed coming from North Dakota and on plants grown from seed coming from Maryland; on plants from good, plump seed and on plants grown from seed rejected by the miller, such as the "rusted wheat" and the "screening wheat" seed. Evidently the nature and first appearance of the rust were determined by local environmental conditions, and variety, age, source, and condition of the seed played a subordinate rôle at least in this particular case. It is also to be noted that the rust appeared at different times on the different cereals except in the case of wheat and rye. This may be regarded as further evidence, if any be required, of the physiological distinction of the *rubigo-vera* forms growing on wheat and rye on the one hand, and on barley and oats on the other.

Hungerford (1920, p. 270) reports an experiment testing the time of first appearance of rust on wheat plants grown from rust-infected and from clean seed, similarly indicating that the condition of the seed does not affect the time of first appearance of the rust.

ROTS OF EARLY STRAWBERRIES IN FLORIDA AND SOUTHERN CALIFORNIA

Neil E. Stevens

(Received for publication July 18, 1921)

The fungi causing rots of strawberries in the southern states have been under investigation since 1915. Each season Florida strawberries have been examined either in the field or in the market by one or more of those interested in the investigation. During several seasons shipping tests were carried on, and the same fruit was examined both before and after shipment. Although there have been found on Florida strawberries fungi belonging to numerous genera, *Rhizopus nigricans* Ehrb. has remained the commonest and most destructive of the fungi causing decay of ripe strawberries from this region.

Four months' study, January to May 15, 1920, of southern California strawberries both in the field and in the market showed a condition quite different from that in Florida. *R. nigricans* appeared to be of distinctly minor importance as a cause of fruit rot of strawberries; whereas a large proportion, frequently over ninety percent, of the rotten berries examined during the four months were affected with a species of Botrytis, probably *Botrytis cinerea* Pers.

There is, then, apparently a marked difference in the relative abundance of these fungi on early strawberries in the two regions, and the probable reasons for this difference are discussed in the present paper. No one would, of course, presume to generalize from the observations of a single season, but so competent and experienced an observer as Dr. H. S. Fawcett of the Citrus Experiment Station at Riverside, California, assures the writer that the extreme prevalence of Botrytis on strawberries is a regular phenomenon of the winter season in southern California.

The regions under consideration include the most southerly important strawberry shipping points on the Atlantic and Pacific seaboards. The strawberry-growing region of central Florida lies east of Tampa, Plant City and Lakeland being the chief shipping points. The southern California strawberry area lies chiefly in Los Angeles county, Gardena and Moneta being large shipping points. Attention will be confined to the early shipping season, during a portion of which these two regions usually dominate their respective markets.

The Fungi

In using the specific names *Rhizopus nigricans* and *Botrytis cinerea*, the writer does not intend to enter upon the debated ground as to whether the fungi so designated constitute good species or collections of species. It is intended merely to indicate that the material studied accords morphologically with the species that have been more or less generally known by those names and produces the characteristic effect on strawberries. It is probable that more than one variety, or species, of each genus occurs on strawberries, and both genera are now being studied by competent investigators, to whom material from strawberries has been supplied.

Both species are widely distributed on a variety of hosts, and there can be no question but that they have long been present in both the areas discussed. The striking difference in their abundance in the two areas must then be due to environmental conditions and not to lack of opportunity for infection. Additional indication that the extreme prevalence of Botrytis in California is due to environmental conditions is found in the fact that Fawcett (7, pp. 207, 209), who has carefully studied Citrus diseases in both Florida and California, finds a similar condition. He reports Botrytis as causing gummosis of trunk and limbs, and a fruit rot of lemons in parts of California, but not in Florida or Cuba.

Host Relations

Commercial strawberry picking in southern California usually begins in March (6, p. 22), although some ripe strawberries may often be obtained throughout the winter, and in very favorable seasons commercial shipments have been made every month in the year. The bulk of the long-distance shipments, however, are made in the month or six weeks following the first general ripening, that is, before the crop ripens in the important strawberry-growing regions of central California. Commercial picking in central Florida usually begins in January, sometimes even before Christmas, and, except when interrupted by frost, long-distance shipments are continued until strawberries ripen abundantly in northern Florida, which is usually in March (5, p. 4). The first four months of the year include, then, the time of earliest picking and the period of important commercial long-distance shipments in both districts.

The standard commercial variety of strawberries in central Florida is the Missionary, in southern California the Brandywine. There is no evidence, however, that the difference in the varieties grown in the two localities influences the relative abundance of the two fungi. Indeed, it has thus far been impossible to demonstrate any varietal resistance to either of these fungi.

With the possible exception of irrigation, which is discussed later, the difference in cultural practice (5, 6) seems to bear no relation to fungous infection.

Climatic Relations

The most striking differences between the areas under discussion are climatic. The climatic differences seem, moreover, to bear a close relation to the difference in the prevalence of the two fungi. Though the writer is responsible for the views on the relation of weather conditions to fungous growth here presented, he has received much assistance in the compilation and interpretation of weather data from the meteorologists in charge of the U. S. Weather Bureau stations nearest the regions discussed, W. J. Bennett of Tampa and H. B. Hersey of Los Angeles.

In comparing the climatic conditions of the strawberry-growing regions of central Florida and southern California, it is necessary to make use, in part, of the meteorological data from Tampa and Los Angeles. These data are, of course, not entirely representative of conditions in the strawberry fields, since the stations are located in cities. The two stations are, however, about equally distant from the strawberry-growing areas, and bear somewhat the same relation to them. Moreover, good thermograph records are available from field stations at relatively short distances from the strawberry regions, and these have been used in comparing temperature conditions. At least the weather data here used represent conditions in the strawberry fields with a degree of accuracy well within the limit of error of such field observations as are here recorded.

Moisture Conditions

The prevalence of Botrytis on strawberry fruits in semi-arid southern California was surprising in view of the fact that in the southeastern states it has been repeatedly observed (10, p. 8) that abundance of Botrytis on strawberries was closely associated with excessive precipitation. The mean annual precipitation at Tampa (49.40 inches) is much greater than that of Los Angeles (15.62 inches). The period under discussion, however (January to April), comes within the "dry season" in Florida and the "rainy season" in California. As shown by the Annual Meteorological Summaries for 1920 published by the two stations (table 1), the mean precipitation for this period as well as for the month of December is actually greater at Los Angeles than at Tampa.

Table 1. *Monthly Mean Precipitation, in Inches*

	Los Angeles, Cal., 1877–1920	Tampa, Fla., 1890–1920
December	2.67	2.05
January	3.34	2.62
February	3.19	2.75
March	2.92	2.20
April	0.89	1.92

Surface irrigation of strawberries is very rare in Florida but is regularly practised in California, a fact which still further increases the amount of water actually used on strawberry beds in the latter state while the berries are ripening.

Soils vary greatly in both regions, but in general the strawberry soils of central Florida are more sandy than those of southern California. The surface of a sandy soil of course dries quickly. Humidity conditions thus seem to be at least as favorable for the development of Botrytis during these months in southern California as in central Florida. If anything, they are somewhat more favorable. In fact, as Bennett has recently shown (2), the region about Tampa has, for so southern a station, a remarkably low relative humidity.

TEMPERATURE RELATIONS

The difference in the temperature of the two regions presents a very interesting correlation with the difference in the temperature relations of the fungi under consideration, and seems to account in large part for the difference in the relative abundance of these fungi on strawberry fruits.

The exact determination of the minimum and optimum temperatures for the growth of an organism is very difficult. Both minimum and optimum temperatures of fungi have been shown to vary with the substratum used and with the duration of the test. It is unlikely, also, that the behavior of a fungus under constant temperatures in the laboratory exactly corresponds to its behavior under changing climatic temperatures. However, its average temperature reactions under maintained conditions furnish our only available basis of comparison with the behavior of a fungus under natural conditions.

Earlier studies (9) of the writer agree with those of Ames (1) and Hanzawa (8), and with the recent results of Brooks and Cooley (4), in placing the minimum for active growth of *Rhizopus nigricans* at about 7.5° to 8° C. (45.5 to 46.4 F.) and the optimum between 30° and 35° C. (86° and 95° F.). In contrast (3), *Botrytis cinerea* will grow somewhat at 0° C. (32° F.) and freely at 2° C. (35.6° F.). Its growth is most abundant at about 25° C. (77° F.) and falls off rapidly at 30° C. (86° F.). Whether these temperatures represent absolute minima and optima for the growth of the two fungi is of little present interest. They may fairly be taken to indicate that the temperatures favorable for the growth of *Rhizopus nigricans* are markedly higher than those for *Botrytis cinerea*.

As yet no thoroughly satisfactory way of expressing the value for plant growth of any given series of climatic temperatures has been devised. Where thermograph records are available, however, the monthly mean hourly temperatures certainly give some indication of the temperature conditions to which an organism is subjected in a given locality. Figures 1 to 4 show the mean hourly temperatures for the first four months of the year at two stations in southern California and two in central Florida.

The curve for Tampa is based on mean temperatures for the years 1910 to 1919 computed by W. J. Bennett, and that for Los Angeles on mean temperatures for the years 1919 and 1920 furnished by H. B. Hersey. The curves for Brooksville, Florida, and Pomona, California, are based on data

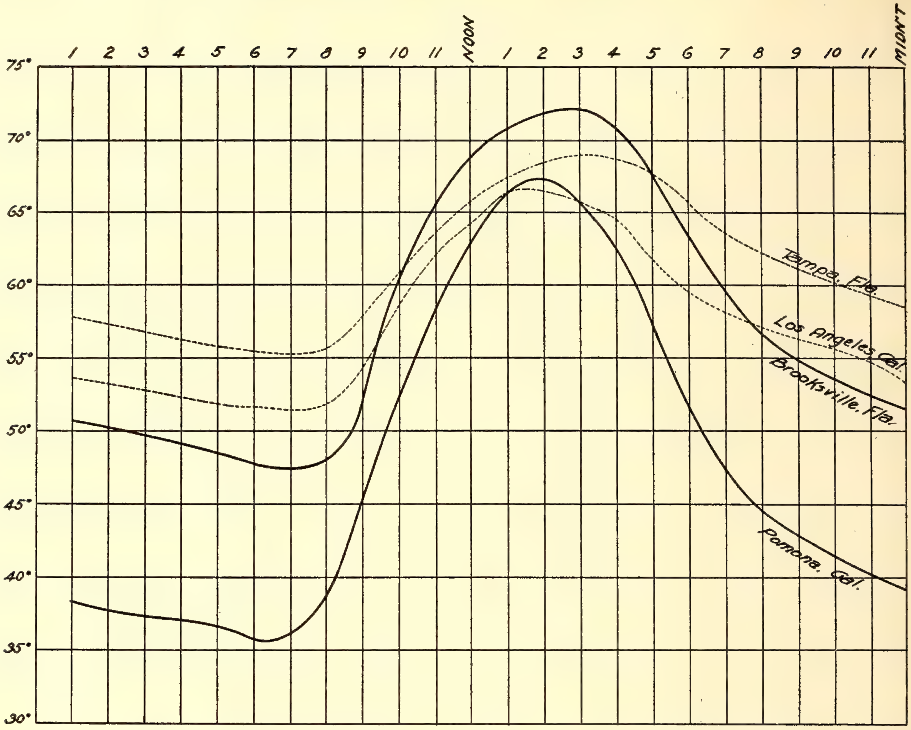


FIG. 1. Mean hourly temperatures for January at Pomona, California (field station), 1919 and 1920; Brooksville, Florida (field station), 1919 and 1921; Los Angeles, California (city station), 1919 and 1920; and Tampa, Florida (city station), 1910 and 1919.

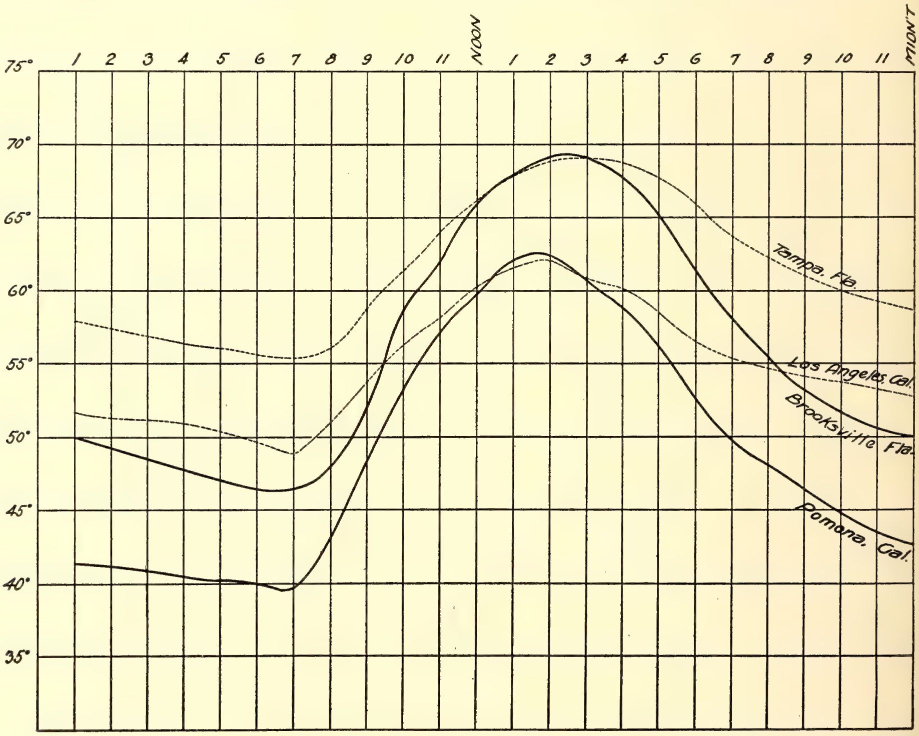


FIG. 2. Mean hourly temperatures for February at stations listed under figure 1.

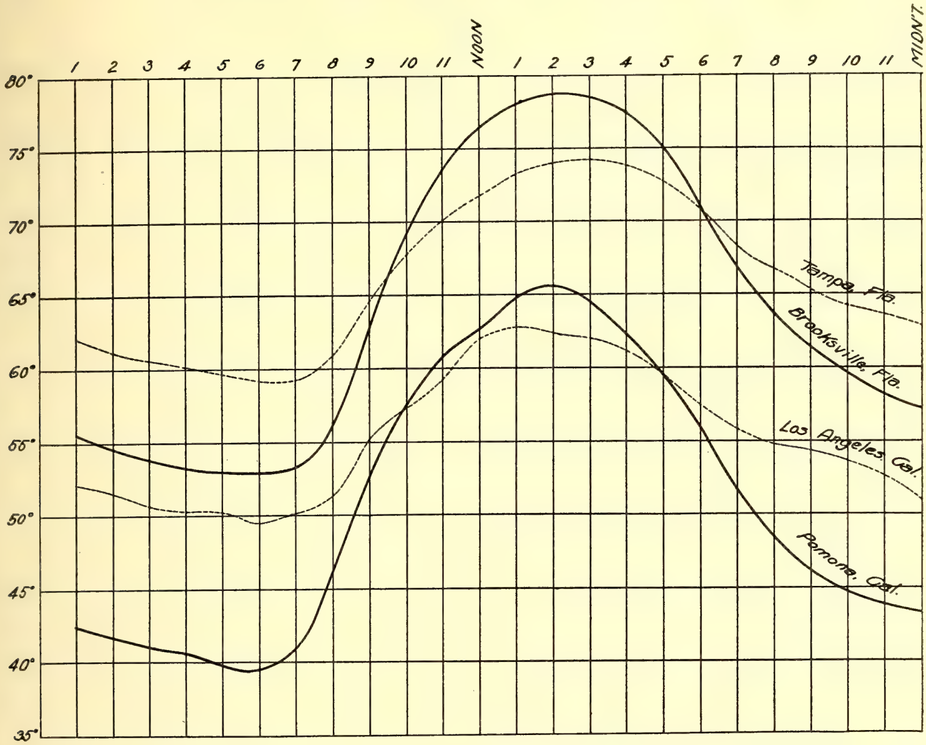


FIG. 3. Mean hourly temperatures for March at stations listed under figure 1.

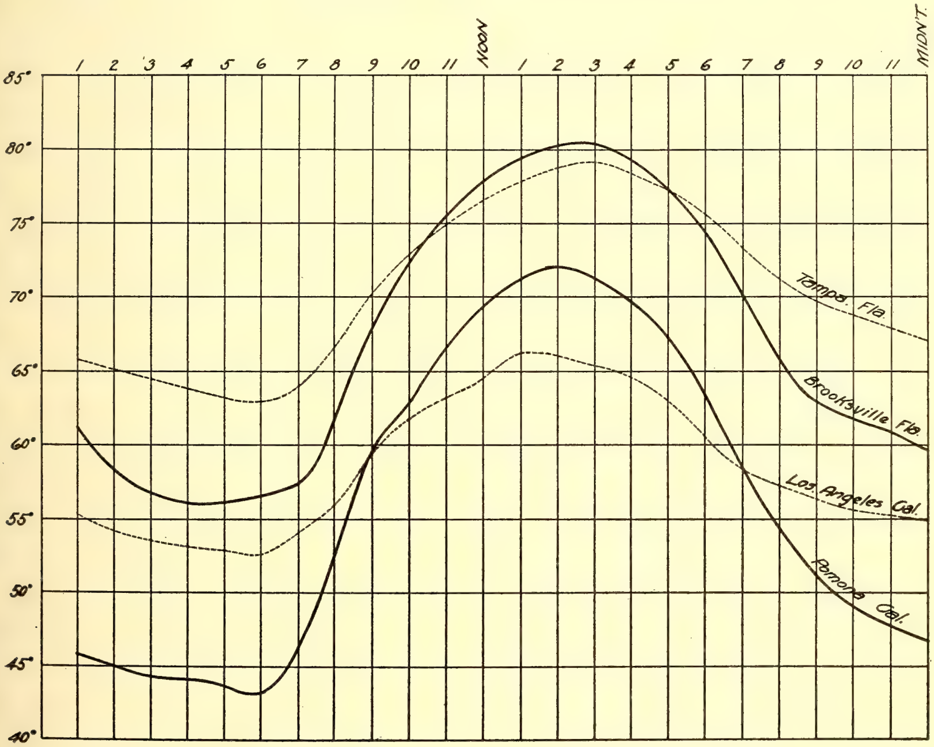


FIG. 4. Mean hourly temperatures for April at stations listed under figure 1.

from original thermograph records corrected by daily maximum and minimum thermometer readings for two seasons (Brooksville, 1919 and 1921; Pomona, 1919 and 1920). While the curves are not strictly comparable, they nevertheless furnish a fairly reliable basis of comparison, since including data for a longer period at the three latter stations would alter the shape of the curves but little. The data for the field stations, Pomona and Brooksville, of course approximate the conditions under which strawberry plants grow more closely than those for the city stations.

The range of mean hourly temperatures in the field in central Florida, January to April, as shown by the curves for Brooksville, is from about 47° F. to 80° F., temperatures known to be favorable for the growth of *Rhizopus nigricans.* In the region about Pomona, California, on the other hand, there is a period each day during which the temperature is less than 47° F., the minimum temperature at which *R. nigricans* grows under most conditions. In January there are about twelve hours out of the twenty-four during which the temperature is below 45° F., in February there are nearly ten, in March nine, and even in April there are five hours each day below this temperature. During this period, more than one third of the day, even in March, *Rhizopus nigricans* is able to make little if any growth. The mean temperature, however, does not go below 35° F., at which temperature *Botrytis cinerea* grows readily. There is thus throughout the winter a daily period, averaging one half the day in January, during which *B. cinerea* grows without competition from Rhizopus.

The writer believes that in this fact is found one of the chief causes of the great prevalence of Botrytis on strawberries and perhaps on other hosts in California during the winter. Under the temperature conditions which prevail in Florida, the rapid growth and abundant spore production of *R. nigricans* enable it to compete successfully with the numerous fungi which are known to infect strawberry fruits. Indeed, it may well be that the temperature of strawberries in Florida is frequently so high as to hinder the growth of Botrytis. Previous studies (11, p. 179) have shown that the temperature of strawberry fruits in the sun is often ten or more degrees C. above that of the air. Under mean shade temperatures of 72° to 80° F., strawberries in the sun may be expected to reach temperatures of 90° to 100° F., which are well above those most favorable for the growth of Botrytis. This may indeed be an important factor, hitherto overlooked, in the favorable influence of rainy weather on the growth of Botrytis. For not only are rainy days generally cooler than clear ones, but on such days the berries remain at or near the temperature of the air, and thus at temperatures more favorable for the growth of Botrytis than the much higher temperatures reached on clear days.

In California, on the other hand, the ability of *Botrytis cinerea* to grow at the lower temperatures gives it an enormous advantage over *Rhizopus nigricans* and over fungi with similar temperature requirements. In the relation of these two fungi on early strawberries, conditions are much like those in refrigerator cars (10, p. 10). That is, temperatures are common under which *Botrytis cinerea* is able to develop readily while the growth of *Rhizopus nigricans* and of fungi with similar temperature requirements is largely inhibited. This partial freedom from competition enables *Botrytis cinerea* to occupy in California a much more important place as a fruit rot of strawberries than in the southeastern United States.

SUMMARY

Rhizopus nigricans, known to be the most common cause of decay of ripe strawberry fruits in Florida, is of minor importance on winter strawberries in California.

Botrytis cinerea, which is common on strawberries only under conditions of extreme moisture in Florida, is regularly abundant in California during the winter.

This difference seems to be due in part to the somewhat more favorable moisture conditions in California, but especially to the fact that during the winter months there is a daily period during which the temperature is favorable for the growth of *Botrytis cinerea*, but unfavorable for the growth of *Rhizopus nigricans*.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D.C.

LITERATURE CITED

1. **Ames, Adeline.** The temperature relations of some fungi causing storage rots. Phytopath. **5**: 11–19. 1915.
2. **Bennett, W. J.** Diurnal variations in humidity. Mo. Weather Rev. **47**: 466–468. 2 fig. 1919.
2a. ——. Humidity and vapor pressure at Tampa, Fla. Mo. Weather Rev. **47**: 710. 1919.
3. **Brooks, C., and Cooley, J. S.** Temperature relations of apple-rot fungi. Jour. Agr. Res. **8**: 139–164. 3 pl., 25 fig. 1917.
4. —— ——. Temperature relations of stone-fruit fungi. Jour. Agr. Res. **22**: 451–465. 1921. [1922.]
5. **Darrow, G. M.** Strawberry culture: South Atlantic and Gulf coast regions. U. S. Dept. Agr. Farmers' Bull. 1026. 40 p. 21 fig. 1919.
6. ——. Strawberry culture: Western United States. U. S. Dept. Agr. Farmers' Bull. 1027. 29 p. 16 fig. 1919.
7. **Fawcett, H. S.** Citrus diseases of Florida and Cuba compared with those of California. Cal. Agr. Exp. Sta. Bull. **262**: 153–211. 24 fig. 1915.
8. **Hanzawa, J.** Studien über einige Rhizopus-Arten. Mycol. Centralbl. **5**: 230–246 257–281. 12 fig. 1914, 1915.
9. **Stevens, N. E., and Wilcox, R. B.** Rhizopus rot of strawberries in transit. U. S. Dept. Agr. Bull. 531. 22 pp. 1 fig. 1917.
10. —— ——. Further studies of the rots of strawberry fruits. U. S. Dept. Agr. Bull. 686. 14 pp. 1918.
11. —— ——. Temperatures of small fruits when picked. Plant World **21**: 176–183. 1918.

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1922

President: Henry C. Cowles
University of Chicago

Vice-President: Margaret C. Ferguson
Wellesley College

Secretary: I. F. Lewis
University of Virginia, University, Va.

Treasurer: Irving W. Bailey
Bussey Institution, Harvard University

Councilors:

N. L. Britton
New York Botanical Garden

J. C. Arthur
Purdue University

C. E. Allen
University of Wisconsin

Any one actively interested in botanical work is eligible for membership. Candidates for membership must be recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including one subscription to the American Journal of Botany. Candidates proposed for membership, and approved by the Council, may receive one copy of the Journal at $4.00 per year pending their election at the winter meeting of the Society.

A Fair And Frank Way To Buy Your Greenhouses

You and I know full well that salesmen have a way of putting the best foot foward, so to speak. In which particular, ours are quite like the rest. They honestly believe we build the best that's built.

Their belief, however, is not based so much on what we have told them as on what they have seen of our houses and our way of doing things.

What they really sell is their confidence in Hitchings & Company.

After which the buyer himself sells the greenhouse to himself.

The shortest short cut to that point of satisfaction, is to go and see some Hitchings houses and talk with their owners.

With that in mind let us send you names of some nearby houses.

We will gladly go with you, however, that is as you may incline.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

PHILADELPHIA
133 So. 12th St.

Vol. IX. MAY, 1922 No. 5

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

CONTENTS

Potato ovules with two embryo sacs . . . . W. J. Young 213
Vegetative vigor of the host as a factor influencing susceptibility and resistance to certain rust diseases of the higher plants II. . . . . M. A. Raines 215
Lignification of mature phloem in herbaceous types. . . . . Carl L. Wilson 239
Observations on the effect of water-raking on the keeping quality of cranberries. H. F. Bergman 245
Incipient drying and wilting as indicated by movements of coconut pinnae. Sam F. Trelease 253
Dr. W. J. Beal's seed-viability experiment. . . . . H. T. Darlington 266
Nutrient solutions for wheat . . . . W. E. Tottingham and E. J. Rankin 270

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
At Prince and Lemon Sts., Lancaster, Pa.
LONDON AGENTS
WHELDON AND WESLEY, Ltd.
28 Essex Street, Strand, London, W. C. 2

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
The Thompson Institute For Plant Research, Inc.

H. H. BARTLETT,
University of Michigan

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ARTHUR H. GRAVES,
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

I. F. LEWIS,
University of Virginia

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Postage to Canada, 20 cents; to other foreign countries (except Mexico and Cuba) 40 cents a volume on annual subscriptions. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume, post free.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or The New Era Printing Co., Inc., Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. 9 MAY, 1922 NO. 5

POTATO OVULES WITH TWO EMBRYO SACS

W. J. YOUNG

(Received for publication July 1, 1921)

Polyembryony, or the presence of two or more embryos in the seed, is of sufficiently uncommon occurrence to excite the interest of botanists whenever found. In some few species it appears as the normal method of seed formation and is found quite regularly, as in the mango and in some of the Citrus fruits. In some varieties of the plum, peach, and other stone fruits, two kernels are often produced in the same pit. This is not true polyembryony, but represents rather two seeds, derived from independent ovules, in the same indehiscent carpel. From the ecological standpoint, however, the two cases are equivalent, as there is no opportunity for the seeds thus formed to become separated in planting.

True polyembryony may arise either by the development of embryos from two distinct eggs contained in separate embryo sacs in the same ovule; or one embryo may arise from the fertilized egg, the others being formed by some vegetative method, being developed from synergids or antipodal cells or formed by the budding of cells lining the embryo sac. The latter method is characteristic of the mango and Citrus fruits already cited. In the former case all embryos show the usual hereditary phenomena of sexual reproduction; in the latter case such phenomena are shown only by the embryo developed as a result of fertilization. The other embryos transmit the characters of the ovulate parent as perfectly as in any other method of vegetative propagation.

It is not always possible to determine on examination of the mature seed by which method the embryos have been formed. In case the tissues of the nucellus persist as a perisperm, embryos produced in separate embryo sacs might be separated by a more or less definite layer of tissue. Embryos produced in the same embryo sac as a rule lie in direct contact, though even in this case there is a possibility of separation by a layer of endosperm. It is thus evident that the determination of the method of origin of the embryos from the anatomy of the seed is often difficult or impossible.

The presence of more than one embryo sac in the ovule is not rare in some of the lower members of the Archichlamydeae, though the condition is very unusual in the Sympetalae and in monocotyledons. It is consequently worth while to record a case which came to the writer's notice in the Irish potato, *Solanum tuberosum* L. In the early development of the

[The **Journal** for April (**9**: 159–212) was issued May 15, 1922]

ovule it occasionally happens that two or more hypodermal cells appear slightly enlarged so that it is difficult to determine from which one the archesporial cell is to be derived. Very soon, however, one cell outstrips the others and gives rise to the embryo sac. While investigating the cytology of the potato, the writer has sectioned some hundreds of ovaries in all stages of development, and the number of ovules examined must have run into the thousands. In only three instances was an ovule found containing more than one embryo sac. Photomicrographs of two of these ovules are shown in figure I.

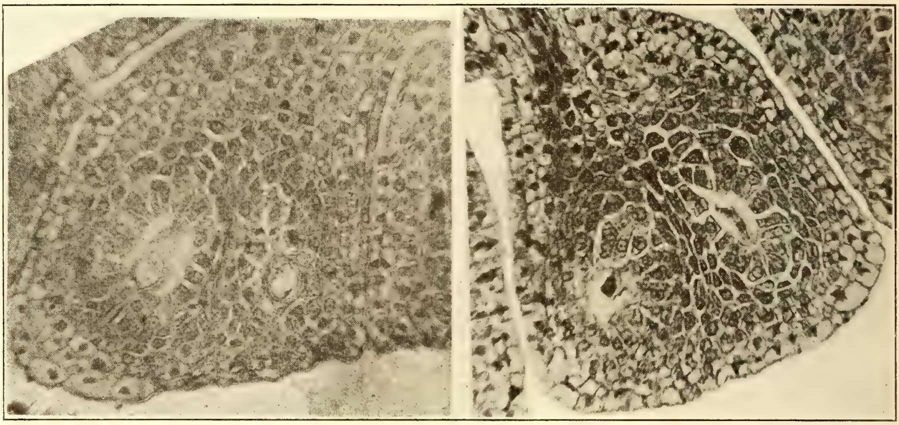


Fig. 1. Photomicrographs of two ovules of *Solanum tuberosum*, each containing two embryo sacs. × 260.

It is a matter of common observation that the potato seldom produces seed, and in the writer's work the germ cells have been found in all stages of degeneration. In the ovules in question degenerative changes were in progress in the embryo sacs, in consequence of which the nuclear structures of the embryo sacs are not well shown. It will be noted that in each case one embryo sac is better developed than the other. The vascular strand supplying the ovule, branches in the funiculus, sending a branch toward either embryo sac. It was observed that the larger branch is given off in the direction of the better developed embryo sac.

Because of the evident rarity of the condition here described, the writer is inclined to regard it as a strictly abnormal occurrence. The affected ovules are believed to have been abnormal from their inception. It is doubtless to be explained as a case of proliferation or doubling, analogous to the hypertrophied condition known as fasciation sometimes observed in stems and other organs.

Department of Horticulture,
Clemson Agricultural College

VEGETATIVE VIGOR OF THE HOST AS A FACTOR INFLUENCING SUSCEPTIBILITY AND RESISTANCE TO CERTAIN RUST DISEASES OF THE HIGHER PLANTS

II

M. A. Raines

(Received for publication July 13, 1921)

Further Field Studies and Experiments

The Rust History of Plots of Cereals Sown at Successive Intervals through the Summer

To test the susceptibility to rust of plants of different ages at each period of the summer and of young plants at different times in the summer, and to follow the subsequent history of the disease on plantings of cereals started at successive intervals through the season, plots of wheat, rye, oats, and barley were started in the breeding plot of the New York Botanical Garden on June 10, June 23, July 6, July 20, August 5, and August 25, 1916. Observations were made at intervals on the height of the plants, the time of first appearance of the rust, the amount of infection, and the proportion of the rust in the teleuto stage.

The results for the different cereals are shown in table 3 and are discussed below. In the table, the days on which the observations were made are given at the top. The height of the plants is given in inches (″). When the plants have headed, it is indicated by an "H." The degree of rust infection is indicated by a numeral, and was estimated on a scale of 10, the values of the numbers in the scale being: 1 = an occasional pustule here and there; most of the leaves not affected. 2 = most of the leaves with from one to five sori. 3 = about ten pustules on each leaf. 4 = leaves heavily infected. 5 = leaves bearing the maximum possible amount of rust. 6 = leaf sheaths infected as well as the leaf blades. 7 = a sprinkling of rust sori on the stem and leaf sheaths; blades heavily infected. 8 = infection on sheath and stem well developed. 9 = heavy infection on the stem. 10 = heaviest possible infection on the whole plant. The teleuto stage is indicated by its Roman numeral, "III." A fraction preceding the "III" gives the proportion of the rust in the teleuto form.

As is indicated in table 3, the plots of wheat planted on June 10 and June 23 headed out in 77 and 64 days respectively. Those planted July 6, July 20, August 5, and August 25 never headed and never exceeded a height of 20 inches. The rust invariably appeared on the young plot as a thin, evenly distributed infection when the host plant was putting out its

TABLE 3

Wheat

Date Sown	Observations								No. of Days to Head
	July 8	July 17	July 24	Aug. 12	Aug. 26	Sept. 15	Oct. 1	Oct. 23	
June 10...	8″–2	12″–4	18″–4	24″–5	H–6	7	7 slight III		77
June 23...	2″	9″–2	12″–4	20″–4	H–5	6 slight III	7 slight III	6 slight III	64
July 6....		5″	8″–1	15″–5	18″–5	18″–5	20″–5	20″–4	
July 20...				10″–3	12″–5	15″–4	20″–5	20″–3	
Aug. 5....					9″–3	10″–4	15″–5	15″–4	
Aug. 25...						5″–2	9″–4	15″–3	

Rye

Date Sown	Observations								No. of Days to Head
	July 8	July 17	July 24	Aug. 12	Aug. 26	Sept. 15	Oct. 1	Oct. 23	
June 10...				destroyed					
June 23...	3″	9″–2	20″–2	H–3	4	7	7	7	33
July 6....		5″	8″–1	15″–3	H–5	6	7	7	51
July 20...				6″–1	12″–4	18″–6	H–7	6	73
Aug. 5....					4″–1	10″–5	15″–6	H–5	79
Aug. 25...						8″–2	10″–5	20″–4	

Oats

Date Sown	Observations								No. of Days to Head
	July 8	July 17	July 24	Aug. 12	Aug. 26	Sept. 15	Oct. 1	Oct. 23	
June 10...	10″	14″	18″	H–2 slight III	6 slight III	8 ¾ III	9 all III		77
June 23...	5″	10″	18″	24″–2 slight III	H–7 ½ III	8 ¾ III	9 all III	9 all III	63
July 6....		4″	8″	15″–2 slight III	20″–6 slight III	H–8 ⅔ III	8 ¾ III	8 all III	70
July 20...				10″–1	12″–3 slight III	18″–5 ½ III	24″–6 ⅔ III	H–8 ¾ III	91
Aug. 5....					9″–1	15″–5 slight III	18″–5 ¼ III	20″–7 ¾ III	
Aug. 25...						6″–1	10″–2	15″–5 ⅓ III	

Barley

Date Sown	Observations								No. of Days to Head
	July 8	July 17	July 24	Aug. 12	Aug. 26	Sept. 15	Oct. 1	Oct. 23	
June 10...	8″	15″	H			2	1		44
June 23...	4″	15″	18″	H		1	3		50
July 6....		3″	8″	15″	H		1	1	50
July 20...				9″	15″	20″	H		70
Aug. 5....					6″	10″	15″	15″	
Aug. 25...						6″	10″	12″	

third leaf. The rust increased steadily in abundance on the leaf blade until it reached the maximum, and only then began to appear on the leaf sheaths. On the stem there were never more than a few scattered pustules. Teleutosori did not appear until the middle of September. They were to be found only on plants of the two oldest plots, and then not without careful search. The observations of October 23 showed a distinct drop in the amount of rust on all the plots of wheat. The new leaf growth of October tended to show but little rust. In view of Johnson's (1912) findings that low temperatures promote uredospore germination, these observations may be interpreted as indicating greater resistance to rust infection on the part of the host tissue due to the decreased rate of metabolic activity consequent on the onset of cooler weather.

All of the plots of rye except that sown August 25 headed out, but the rate of growth varied as is indicated by the successively greater intervals required by the younger stands to head out. The behavior of the rust on the plots of rye was much the same as on the wheat. In the younger plantings it was somewhat less marked and less severe than on the wheat plants of the same age, but the development of the rust was more severe on the rye than on the wheat. It seemed, too, to go more readily to the leaf sheaths and stems in the case of the rye. No teleuto was found on the rye.

Of the oats, the first three plots headed out in 77, 65, and 70 days respectively; the fourth plot produced only one head, 91 days after planting. The plots planted August 5 and August 25 never headed out. No rust appeared on the oats until August 12 when four plots were up, ranging in age from seedlings in the four-leaf stage to plants in bloom. The rust appeared on all four of the plots at the same time and in relatively the same abundance. However, once the rust had appeared, its subsequent history on the various plots differed decidedly. The older the plant, the greater the abundance of rust on it, and the larger proportion of the rust in the teleuto stage.

In the series of barleys, the three older plots headed in 42, 50, and 50 days respectively. The fourth put out a few heads 70 days after planting.

The last two sowings, of August 5 and August 25, never reached the heading-out stage. The barleys were rust-free until the middle of September, when a few uredo pustules were to be discovered on the leaves and sheaths of the three older plots. No teleuto was found.

As has been noted for the plots of wheat and rye, even more strikingly in the case of the oats, the rate of development of the parasite differs with the host. It is much more rapid in the case of the oats than in that of the other cereals. For example, in the seedling stage the amount of rust on the plants may appear less on the oats than on the rye and wheat, although at the time of heading out the same plants will show the reverse condition, the oats being much more severely infected.

The rust history of plots of cereals sown at successive intervals through the summer may be taken as indicating that the age and maturity of the host is a factor in the progress of the disease, and that the action of this factor differs with the identity of the host plant.

Laboratory and Greenhouse Studies

Culture Methods

Four cereal rusts—*Puccinia coronifera* Kleb., *P. secalina* Grove, *P. triticina* Eriks., and *P. Sorghi* Schw.—were successfully grown for periods of time on the host in pots on greenhouse benches as described by Melhus (1912) and Fromme (1913), and under aseptic conditions on host seedlings growing in test tubes as described by Ward (1902*a*) and Mains (1917). Variations were introduced in both methods.

Fromme (1913) reviews the problem of growing cereal rusts in the greenhouse. The method recommended by him includes sowing rust spores on new host plants every few weeks by applying them with a scalpel or camel's hair brush, or spraying on in suspension in water with an atomizer, and then putting the host plants into a moist chamber for from 24 to 48 hours to provide the conditions of high humidity necessary for spore germination and infection. Tests, however, indicated that the first part of the method recommended by Fromme, artificially sowing rust spores on the new host, was not necessary under the conditions obtaining in the Columbia greenhouse. It was found that when new host plants are grown beside infected plants in the greenhouse, rust spores will be sown on them by natural agencies, such as convection and other atmospheric currents, sufficient to produce abundant infection if conditions of high humidity are provided occasionally to render possible the germination of the spores.

Accordingly, the method adopted for maintaining stock cultures of the cereal rusts in the greenhouse was to introduce new host plants alongside the infected plants every third week and to cover the cultures with a moist chamber every second or third night. The fungus maintained itself self-sown in this manner, and no artificial inoculations were needed. The advantage of such a method of maintaining stock cultures of cereal rusts is that it eliminates the most technical operation, that of sowing or applying the fungous spores to the new host, and reduces the problem of maintaining cereal rusts in culture in the greenhouse to a non-technical routine such as can be entrusted to the average gardener or greenhouse man.

In growing the rust under aseptic conditions on seedlings in test tubes, the method developed was to treat the seed with chlorine water (*cf.* Wilson, 1915), put the seed to germinate on filter paper in Petri dishes, and transfer the germinated seed to a test tube plugged with cotton. Half an inch of sterile water was put into the test tube with the plant. The reserve food materials of the endosperm are capable of bringing the seedling to the third leaf stage, which is sufficient to raise a generation of the rust on it. *P. coronifera* was cultivated for 10 generations in this manner, transfers being made once a month; *P. Sorghi* for 8 generations; *P. triticina* for 8 generations; and *P. secalina* for 6 generations. A small platinum spatula was employed for making transfers; spores were applied to the upper surface of the first leaf in each case, and material for inoculum was taken from the under surface. That a cereal rust can thus be grown under conditions free from accidental contamination was indicated by the total absence of organic growth, bacterial or fungous, when a rust-infected seedling was deposited on sterile beef-peptone agar.

Studies on the Incidence of Infection by Measured Doses of Uredospores of Puccinia Sorghi on Zea Mays

An effort was made to determine the minimal dose of uredospores of *P. Sorghi* that (1) can possibly, and (2) will certainly produce infection in *Zea Mays.* 191 tests were made on corn seedlings growing under aseptic conditions in twelve-inch test tubes.

The method employed to determine the dose and to inoculate was as follows: A dilute suspension of uredospores was made in a vial of sterile water. A small drop from this suspension was put on a piece of sterile cover slip, and the number of uredospores in the drop was counted under the microscope. The piece of cover glass was then inverted and deposited on the upper surface of the first leaf of the young corn seedling in the test tube, about one half inch below the tip, bringing the drop of water containing the known number of uredospores in contact with the host tissue. The work was done in the winter in the laboratory, with no rust growing free anywhere in the building, so that the danger of accidental contamination was negligible. No infection ever developed on the seedlings except on the spot where the plant had been inoculated.

The inoculated seedlings were kept under observation for 21 days. If the inoculated leaf yellowed or withered before 15 days, the plant was discarded. The data on 191 tests are shown in table 4.

TABLE 4

No. of Spores	No. of Inoculations	No. of Infections	No. of Spores	No. of Inoculations	No. of Infections
1	58	2	52	1	0
2	18	0	53	1	0
3	6	1	55	3	0
4	4	0	56	1	0
5	10	0	57	1	1
6	4	0	58	1	0
7	3	0	59	1	1
8	5	1	61	1	0
9	3	1	62	1	0
10	6	1	63	1	0
11	2	0	64	3	0
12	2	0	65	1	0
13	2	1	67	1	0
14	2	0	68	2	1
16	2	1	69	1	0
19	1	0	72	1	1
22	1	0	74	1	0
23	1	0	77	2	2
25	2	2	79	1	0
27	1	0	86	1	0
28	3	1	98	1	0
31	1	0	100	1	1
32	1	0	103	1	1
35	1	0	104	1	0
36	2	1	119	1	1
40	3	0	142	1	1
42	3	0	135	1	1
44	2	0	425	1	1
47	1	0	700	1	1
48	3	0	800	1	1
50	1	0	1000	1	1

The results cannot be considered as entirely convincing in view of the many factors involved in a successful inoculation. The evidence indicates, however, that, as to the minimal number of uredospores which can possibly produce infection, it is possible for a single uredospore to produce infection. In each of the two cases in which infection was produced after inoculation with a single uredospore, the rust appeared after the usual incubation period as a very small pustule at the point of inoculation. In one case the infected leaf withered shortly after the appearance of the pustule; in the other instance the mycelium showed normal growth, and about a week later seven new uredosori were produced in a ring around the first pustule.

As regards the second question, the minimal dose of uredospores which will certainly produce infection, the data indicate this to be, for the conditions under which the work was done, between 100 and 125. This is high. In view of the varying viability of spores taken at one time from the same pustule, and of their further variation with the age of the pustule, it was not considered possible actually to test the germination for each sample used. However, the spores were always taken from the surface of a large and pulverulent sorus, and, considering that the index of germination of the uredospores under the conditions of inoculation was from 75–90 percent, and that it is possible for a single spore to produce infection, we can say that (taking the conservative germination figure of 50 percent) of more than fifty spores germinating on the surface of the leaf, only one produced successful infection. Evidently, successful infection by a uredospore involves other factors besides that of germination on the leaf surface of the host plant.

The Constitution of the Fungous Mycelium as a Factor in Teleutospore Production by Puccinia coronifera

Our knowledge of the conditions governing teleutospore production in the cereal rusts is summarized and extended by Gassner (1915), who considers that the determining factor is the physiological aging of the host tissue, teleutospore production being particularly coincident with the mobilization of the food resources of the plant for flower and fruit production. The picture of teleutospore production presented by the plants of the experimental field plots described above closely parallels Gassner's observations in similar experiments and is consistent with his views.

Consideration of the behavior of *Puccinia coronifera* as regards teleutospore production, when grown in the greenhouse, leads to the suggestion that the protoplasmic constitution of the fungous mycelium may be a factor. Greenhouse cultures of the rust from material brought in from the field in the vicinity of New York exhibited moderate teleutospore production. A series of cultures from material sent the writer by J. I. Durrell from Ames, Iowa, on the other hand, grown at the same time on similar host material and under similar conditions, showed very abundant teleutospore production, the difference in this respect between the two series of cultures being readily noticeable. While such teleutospore production on potted oat seedlings in the greenhouse is more commonly on the older infected leaves, which are yellowing at the tip, it is not unusual to observe the production of teleutospores by rust pustules on young and vigorous leaves shortly after first infection.

Experiment showed that it is readily possible to secure variation in the tendency of the rust towards teleutospore production by selection. The rust was grown in test tubes under aseptic conditions. Large variation in the tendency towards teleutospore production was noted in cultures of the third generation, some rust cultures showing no teleutosori at all; in others as much as 75 percent of the pustules were teleutosori. Two series of cultures were therefore propagated. In one of the series, transfers were made from cultures showing no teleutospores. Of 36 cultures in this series, 20 showed complete absence of teleutosori; only 2 of the cultures developed more than 50 percent of the winter stage.

In the second series, transfers were made from cultures showing 75 percent teleutosori. Of 35 cultures in this series, 30 showed more than

50 percent teleutosori, and only 5 less than that. Two of the cultures in this series never produced any uredospores whatever, teleutosori only being developed. It was obviously impossible to make transfers from such cultures.

Figure 3, Plate XII, shows a rust mycelium in which only the first pustule produced uredospores, succeeding pustules bearing only teleutospores.

Apparently there may be wide differences in the tendency towards the production of teleutospores in different cultures of a rust fungus, and the factor of fungous constitution should be given consideration in work on the conditions of teleutospore production.

Nutrition and Growth Studies

Water Cultures

Six experiments were performed with *Puccinia Sorghi* on corn to test the effect on rust development of growing the host plants in culture solutions of varying nutritive value. A sugar corn was used, as being more susceptible to rust than a flint or dent corn. The seedlings were grown in water culture in 250-cc. Erlenmeyer flasks. Knop's nutrient solution was used as a base. Except as otherwise noted, the endosperm was removed about the time that the first leaf was breaking through the coleoptile, so that the plant was entirely dependent for sustenance on the mineral salts it could obtain from the nutrient solution and on the carbohydrates it could manufacture in its leaf tissue. Inoculation was effected by spraying with a suspension of uredospores and covering with a bell jar for 24 hours. Observations were made on the incubation period and on the progress of the disease on the plants. The dry weight of the top of the plant at the conclusion of the experiment was taken as an index of the relative vigor of growth of the plant.

TABLE 5

Exp. 1. Effect of Renewing Solution. Plants Inoculated February 28, 1919

No. of Plant	Treatment	Dry Weight of Top of Plant in Mg.	Observations		
			March 9	March 11	March 13
1.....	Solution changed once a week	340	3 pustules on third leaf	1 pustule on second leaf; 3 pustules on third leaf	2 pustules on second leaf; 3 pustules on third leaf
2.....	Solution not changed	310	No infection	1 pustule on second leaf	1 pustule on first leaf; 4 pustules on second leaf; 1 pustule on fourth leaf
3.....	Solution not changed	280	No infection	No infection	1 pustule on first leaf; 1 pustule on third leaf

Exp. 2. Effect of Removing Endosperm. Plants Inoculated February 28, 1919

No. of Plant	Treatment	Dry Weight of Top of Plant in Mg.	Observations			
			March 9	March 11	March 13	March 15
1.....	Endosperm not removed	150	3 pustules on first leaf	4 pustules on first leaf	4 pustules on first leaf	5 pustules on first leaf
2.....	Endosperm removed	80	No infection	1 pustule on second leaf	1 pustule on second leaf	3 pustules on second leaf
3.....	Endosperm removed; solution rendered highly toxic by large excess of Fe_2Cl_6	40	No infection	No infection	No infection	No infection

Exp. 3. Effect of Culture Solutions of Varying Nutritive Value. Plants Inoculated March 19, 1919

No. of Plant	Treatment	Dry Weight of Top of Plant in Mg.	Observations			
			March 25	March 26	March 27	March 29
1.....	Full nutrient solution; endosperm not removed	160	Infection on second and third leaves	Infection on second and third leaves	13 pustules on second leaf; 4 pustules on third leaf	2 pustules on first leaf; 1 pustule on second leaf; 7 pustules on third leaf
2.....	Full nutrient solution	80	No infection	No infection	3 pustules on first leaf; 4 pustules on second leaf	5 pustules on first leaf; 5 pustules on second leaf
3.....	Full nutrient solution	80	No infection	No infection	1 pustule on first leaf; 2 pustules on second leaf; 1 pustule on third leaf	3 pustules on first leaf; 4 pustules on second leaf; 2 pustules on third leaf
4.....	Nutrient solution rendered toxic with excess of Fe_2Cl_6	50	No infection	No infection	2 pustules on second leaf	2 pustules on second leaf; 1 pustule on third leaf
5.....	Tap water	40	No infection	No infection	1 pustule on first leaf; 2 pustules on third leaf	2 pustules on first leaf; 2 pustules on second leaf; 1 pustule on third leaf
6.....	Distilled water	20	No infection	No infection	No infection	2 pustules on second leaf
7.....	Highly toxic nutrient solution	20	No infection	No infection	No infection	1 pustule on first leaf

Exp. 4. Effect of Varying Concentration of Nutrient Solution. Plants Inoculated April 2, 1919

No. of Plant	Concentration of Nutrient Solution	Dry Weight of Top of Plant in Mg.	Observations		
			April 8	April 9	April 14
1.....	0.012	20	No infection	No infection	No infection; plant dying
2.....	0.009	140	Rust showing on second leaf	12 pustules on second leaf	Large number of pustules on first, second, and third leaves
3.....	0.006	110	No infection	3 pustules on second leaf	5 pustules on second leaf; plant dying
4.....	0.003	110	Rust showing on third leaf	19 pustules on second leaf; 10 pustules on third leaf	5 pustules on first leaf; 20 pustules on second leaf; 10 pustules on third leaf
5.....	0.0015	100	No infection	No infection	17 pustules on first leaf; 8 pustules on second leaf
6.....	Distilled water	(destroyed)			

In all the water-culture experiments (table 5) an increase in the incubation period of the rust with depression in the vigor and rate of growth of the host plant was apparent. Coincident with the increased incubation period of the rust on host plants of poor growth and little vigor went always a marked depression in the luxuriance of the fungus. The pustules were appreciably smaller, and produced decidedly fewer spores.

Comparing the incubation period of the rust on the leaves of the same plant, it is found to be shorter on the younger leaves. Comparing the first and second leaves, we find:

Infection noted on the first leaf before it appeared on second leaf	0
Infection noted simultaneously on first and second leaves	5
Infection noted on second leaf before first	7
Infection on first leaf; none on second	2
Infection noted on second leaf; none on first	7

The older host tissue, it would seem, provided a less congenial environment for the development of the rust.

Incidental to the above-described water-culture experiments was the demonstration of the ability of the rust to develop on chlorotic tissue. Some corn seedlings were grown in iron-free nutrient solution, and the fourth and fifth leaves produced by the plants were completely blanched. The plants were sprayed with a spore suspension to test the susceptibility of these leaves to the rust. Nine days after inoculation the chlorotic leaves showed abundant rust infection. Giddings (personal communication) has obtained infection with *Gymnosporangium juniperi-virginianae* on apple leaves blanched by being kept in the dark room while unfolding from the bud. It may be concluded that the presence of chlorophyll is not a necessary condition for rust development.

Soil Cultures

From the studies of Sheldon, Ward, and Stakman, as also from the experiments described above, it may be considered as established that, within the range of forms worked with, conditions unfavorable to the growth of the host cause an increase in the incubation period of the rust and depress the luxuriance of growth of the fungous mycelium as indicated by the size of the pustules and the number and size of the spores produced in them.

Concerning the effect of conditions unfavorable to the growth of the host on the incidence of rust infection—the number of successful infections produced on a unit area of host tissue by a given dose of inoculum—our knowledge must be regarded as not so definite. The data extant are subject to criticism because of the relatively small number of variables studied and because of the irregularity of dosage inherent in the method of inoculation employed. Ward (1902*b*) applied spores to the leaf by means of a swab of cotton, and Stakman (1914, p. 11) employed a flat inoculating needle for this purpose.

Studies on the relation between host vigor and incidence of infection, to be of critical value, must be made with numbers of variables sufficient to preclude undue distortion of the results by fluctuations in condition of host and fungus, and by errors in the taking and studying of data; the method of inoculation employed must stand criticism as to the uniformity of dosage for the variables compared; and, if any but the grossest relations between the variables studied are to be made apparent, a more exact basis than visual observation and judgment must be employed for determining vigor of growth of host plant and degree of rust infection on it.

In the experiments described below on the relation between host vigor in the oat plant and its susceptibility to crown rust, data were obtained on 1450 individual plants receiving different nutritive treatment and exhibiting wide variation in vigor of growth. The plants were grown in pots in the greenhouse. Inoculation was effected under natural field conditions by placing the pots containing the experimental plants out of doors near a stand of oats heavily infected with crown rust. Analysis of the data indicates that the dosage for the variables compared was uniform. The experiments were concluded and the readings taken before the rust on any of the plants approached the maximum that the leaf tissue could support, so that the infection present at the time may be considered an index of the response of the host tissue to the conditions of inoculation to which it was subjected, and variation in this response between host tissues receiving similar doses of inoculum was presumably due to differences in the condition of the tissues compared.

Values for the vigor of the host plant and for the amount of rust infection present on it were obtained as follows: At the conclusion of the experiment the plant was cut off at the base and observations were taken of the number of rust pustules on the upper surface of each leaf, of the length of each leaf in inches, of the extreme length of the entire plant, and of the number of stools it had produced. The plant was then dried, and its dry weight was obtained. The dry weight of the plant was adopted as the index of its relative vigor of growth, because more accurate seriation of the variables is possible on this value than on an index such as the height of the plant or the total leaf length.

As an index of the degree of infection of the plant the value adopted was the number of pustules on an average unit area of the most severely infected leaf—calculated by dividing the number of rust pustules on the leaf by the length of the leaf in inches, and by its width at the base in sixteenths of an inch. This value was found to have a positive correlation ($r = .7803 \pm .0167$ for the 250 variables of experiments I, II, and III) with the value that at first thought would seem most desirable: namely, the total number of rust pustules counted on the leaves of the plant, divided by the total leaf length in inches, and by the largest leaf width in sixteenths of an inch—and is preferable for adoption in work of this kind not only because it is easier to obtain, but also because it avoids the error introduced by the development of new leaf surface during the incubation period of the rust. The most highly infected leaf on the plant was usually the lowest leaf in good condition. In tables 6–10 both values are given.

Experiment I

66 oat plants were grown in soil in 2-inch pots, divided into three groups on the basis of the number of plants grown to a pot. The soil was a rich garden loam. The seed was sown July 6, 1920, three grains being put into the soil for every plant desired, and the seedlings were later thinned out to the number of plants desired. The pots were kept on a bench in the greenhouse until August 11, when they were taken out of doors and set near a patch of rusty oats, subjecting the plants to natural conditions of inoculation and infection. The experiment was concluded on August 24. The data on this experiment are given in table 6.

TABLE 6

Group	No. of Variables	No. of Plants to a 2-inch Pot	Mean Dry Weight of Top of Plant in Mg.	Mean No. of Pustules per Ave. Unit Area of Total Leaf Surface	Mean No. of Pustules per Ave. Unit Area of most Severely Infected Leaf
a	23*	5	61	1.1	4.2
b	18	2	135	2.1	6.0
c	25	1	183	2.3	6.1

* Plus 2 destroyed.

Experiment II

70 plants were grown in 3-inch pots, divided into three groups on the basis of the number of plants grown to a pot. Soil, method of seeding, and dates

of sowing, setting out of doors to be inoculated, and of concluding the experiment were the same as in experiment I described above. The results are shown in table 7.

TABLE 7

Group	No. of Variables	No. of Plants to a 3-inch Pot	Mean Dry Weight of Top of Plant in Mg.	Mean No. of Pustules per Ave. Unit Area of Total Leaf Surface	Mean No. of Pustules per Ave. Unit Area of most Severely Infected Leaf
a	25	5	96	1.6	4.1
b	20	2	264	1.7	4.8
c	25	1	407	2.0	5.2

Experiment III

120 plants were grown in 4½-inch pots, divided into four groups on the basis of the number of plants grown to a pot. Soil, method of seeding, and dates of sowing, of setting out-doors to be inoculated, and of concluding the experiment were the same as in experiment I. Results are given in table 8.

TABLE 8

Group	No. of Variables	No. of Plants to a 4½-inch Pot	Mean Dry Weight of Top of Plant in Mg.	Mean No. of Pustules per Ave. Unit Area of Total Leaf Surface	Mean No. of Pustules per Ave. Unit Area of most Severely Infected Leaf
a	50	10	161	1.5	3.6
b	25	5	352	2.4	6.4
c	20	2	661	2.7	6.5
d	25	1	976	2.5	6.2

Experiment IV

600 oat plants were grown in 4½-inch pots, 5 plants to a pot; 15 grains being planted in each pot in the first place, and the young seedlings thinned out to the desired number. The plants were divided into six groups of 100 individuals each on the basis of soil composition and treatment, as follows:

Group A: Soil composed of sand only.

Group B: Soil a mixture of ⅘ sand and ⅕ garden loam.

Group C: Soil a mixture of ½ sand and ½ garden loam.

Group D: Soil the same mixture as in Group C. In addition, KCl at the rate of 350 pounds to the 6-inch acre of 2,000,000 pounds was intimately mixed with the soil.

Group E: Soil the same mixture as in Group C. In addition, acid phosphate at the rate of 750 pounds to the acre was intimately mixed with the soil.

Group F: Soil the same mixture as in Group C. In addition, sodium nitrate at the rate of 500 pounds to the acre was intimately mixed with the soil.

Seed was sown July 11, 1920. On July 24, July 31, and August 7 the plants of groups *D*, *E*, and *F* had additional quantities of fertilizer applied in water solution at the rate of 100 pounds to the acre. On August 9 the plants were placed out of doors to be inoculated. The experiment was concluded on August 27. Figures 1 and 2, Plate XI, illustrate the growth differences obtained between the plants of the different groups in these experiments.

In table 9, the groups are arranged in order of the vigor of growth exhibited by the plants.

TABLE 9

Group	No. of Variables	Soil Treatment	Mean Dry Weight of Top of Plant in Mg.	Mean No. of Pustules per Ave. Unit Area of Total Leaf Surface	Mean No. of Pustules per Ave. Unit Area of most Severely Infected Leaf
A	100	sand	120	.6	2.0
E	100	acid phosphate	155	.8	2.7
B	100	$\frac{4}{5}$ sand	200	.7	2.3
D	100	KCl	202	1.0	3.2
C	100	$\frac{1}{2}$ sand	341	.9	3.1
F	100	$NaNO_3$	564	1.2	4.5

Experiment V

This was a duplicate of Experiment IV, started a week later. The seed was sown July 17, the plants were placed out of doors to be inoculated August 10, and the experiment was concluded August 31. The results are shown in table 10.

TABLE 10

Group	No. of Variables	Soil Treatment	Mean Dry Weight of Top of Plant in Mg.	Mean No. of Pustules per Ave. Unit Area of Total Leaf Surface	Mean No. of Pustules per Ave. Unit Area of most Severely Infected Leaf
A	100	sand	47	5.1	11.6
B	100	$\frac{4}{5}$ sand	53	5.1	11.8
E	100	acid phosphate	93	5.2	11.3
C	100	$\frac{1}{2}$ sand	93	4.5	9.5
D	100	KCl	96	4.4	10.2
F	100	$NaNO_3$	359	3.1	7.6

Relation between Host Vigor and Pustule Size

In all five of the soil-culture experiments there was evident a marked decrease in the size of the rust pustules on the host plants the growth rate of which was depressed. The lengths of 100 contiguous pustules on plants from groups *a* and *c* of experiment I; groups *a* and *c* of experiment II; groups *a* and *d* of experiment III; groups *A* and *F* of experiment IV; and groups *A* and *F* of experiment V were found to fall into the classes shown in table 11.

TABLE II

	Experiment									
	I		II		III		IV		V	
	Group		Group		Group		Group		Group	
	a	*c*	*a*	*c*	*a*	*d*	*A*	*F*	*A*	*F*
No. of pustules ½ mm. long......	94	20	72	14	82	22	96	12	70	8
" " " 1 mm. "	6	20	28	34	18	30	4	30	25	24
" " " 1½ mm. "		34		38		30		46	3	24
" " " 2 mm. "		24		8		12		8	2	34
" " " 2½ mm. "		2		4		4		4		7
" " " 3 mm. "				2		2				3
Sum of lengths of 100 pustules in mm.	53	134	64	130	59	126	52	131	68	169

Figures 4 and 5 of Plate XII illustrate the relative size of the pustules on leaves of semi-starved and of vigorously growing plants.

Pustules attained a larger size on the more rapidly growing host plants, indicating that a more luxuriant host tissue means a more luxuriant parasitic mycelium.

DISCUSSION

Relation between Host Vigor and Incidence of Infection

On their face the figures obtained in the soil-culture experiments indicate that in experiments I, II, III, and IV there occurred a decreased incidence of infection with depression in growth vigor of the host; but in experiment V the figures indicate quite as definitely precisely the opposite relation—namely, increased incidence of infection with depression in the growth rate of the host.

The dosage for all six groups of variables in soil-culture experiments IV and V was probably essentially the same. The plants were arranged in order of alphabetical designation of the groups: *A*, *B*, *C*, *D*, *E*, *F*. The possibility might be suggested that in experiment IV inoculation proceeded from the direction of *F* and that the plants from *F* to *A* were subjected to progressively diminishing doses of inoculum; and, conversely, that in experiment V inoculation was from the direction of *A* and that the plants from *A* to *F* received progressively diminishing doses of uredospores. This would make the amount of infection observed on the plants of the different groups a function of their positions relative to each other. But actually the amount of infection observed is correlated not with the position of the group but with its relative growth vigor as indicated by the mean dry weight of the plants. Thus, in both experiments IV and V, group *E* exhibits an amount of infection not like group *F*, next to which it was placed, but like group *B* which it resembles in vigor of growth. We may conclude that the dosage for the variables compared in any experiment was uniform and that the variation in the amount of rust observed on the different groups of plants in the experiment is due to differences in the reactions of the plant tissue to the infection to which they were subject.

The explanation of the apparent reversal of the result in soil-culture experiment V as compared with the others is probably to be found in the age of the plants and in the length of time they were exposed to infection. The experiments are compared in table 12.

TABLE 12

Experiment	I	II	III	IV	V
Age of plants at conclusion of experiment (days)	49	49	49	47	45
Age of plants when set out of doors to be inoculated	35	35	35	31	24
Number of days out of doors and exposed to infection	15	15	15	16	21
Number of variables	66	70	120	600	600
Average dry weight of top of plants (mg.)	127	278	538	264	123
Average infection (total leaf surface)	1.8	1.8	2.3	.9	4.6
Average infection (most severely infected leaf)	5.4	4.7	5.7	3.0	10.4

Experiment V differs from the other four experiments in that (1) when set out of doors to be inoculated the plants were from 7 to 11 days younger. Even at the conclusion of the experiment these plants had only half the dry weight of the plants of experiment IV and were evidently much less mature. (2) When the experiment was concluded the plants had been out of doors and subject to infection 6 days longer. If we allow an incubation period of 10 days for the rust, then the rust present on the plants of experiment V at the conclusion of the experiment represents inoculation through a period of time twice as long as in the case of the other experiments. (3) The amount of rust on the plants at the conclusion of the experiment was several times greater in experiment V than in any of the other experiments.

The last-mentioned fact immediately brings into view an aspect of the method of experimentation used tending to limit the value of the pustule count as a criterion of the frequency of penetration and infection by the uredospore germ tube. It is probable that only in cases of very sparse infection is there a pustule for every focus of infection, and that only in cases of very sparse infection is the number of pustules counted an accurate index of the number of infections which have taken place. With abundance of infection there appears a tendency for the coalescence of foci of infection, for two or more mycelia the result of contiguous infections to coalesce and produce only one pustule; and this tendency would be highly accentuated on the more vigorously growing host plants where the parasite finds a favorable nidus and develops more luxuriantly. In experiment V the error introduced by the coalescence of mycelia may well have masked a higher incidence of infection in the vigorously growing plants of group *F* and have converted it into an apparently lower susceptibility. It is noticeable that the pustules were larger in experiment V than in the other four experiments.

Variation in the Incidence of Rust Infection with Variation in the Growth Vigor of the Host Plant, due to Constitutional or Racial Differences

In soil-culture experiments IV and V, when the 100 variables of each group were arranged in the order of their dry weight, the series divided into five equal parts of 20 variables each, and the average weights and degrees of infection of these sub-groups determined, a certain relation was apparent between the relative weight attained by a plant and the incidence of rust infection on it. The figures obtained in this analysis of the data are presented in table 13.

TABLE 13

Experiment IV

Group	Mean Dry Weight of Tops of Plants in Mg.					Mean Infection per Unit Area of most Severely Infected Leaf of Plant				
	Sub-groups									
	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth
A. . . .	40	65	110	148	239	2.3	2.4	1.8	2.0	1.6
E. . . .	99	129	148	172	231	2.8	2.1	2.9	3.5	2.1
B. . . .	99	140	179	231	345	2.3	2.9	2.5	2.1	1.8
D. . . .	90	144	182	231	363	3.6	3.0	2.5	3.9	2.9
C. . . .	141	233	336	430	596	4.0	3.0	2.3	4.4	1.7
F. . . .	305	450	555	653	812	4.7	5.7	5.2	3.8	3.3
	129	193	252	311	431	3.3	3.2	2.9	3.3	2.2

Experiment V

Group	Mean Dry Weight of Tops of Plants in Mg.					Mean Infection per Unit Area of most Severely Infected Leaf of Plant				
	Sub-groups									
	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth
A. . . .	21	34	43	54	83	11.8	13.6	11.5	11.6	9.3
B. . . .	22	36	50	65	92	12.3	14.0	11.7	12.2	8.6
E. . . .	42	67	85	108	159	15.9	12.2	11.9	8.6	7.8
C. . . .	53	71	88	106	148	10.2	10.0	11.0	8.6	7.4
D. . . .	47	72	90	107	162	12.0	13.2	10.5	8.0	7.2
F. . . .	190	283	349	416	558	9.9	6.8	7.2	7.7	6.2
	63	94	118	143	200	12.0	11.6	10.6	9.5	7.8

The figures show that there was considerable variation in the growth attained by plants receiving the same treatment, and that the larger plants were less susceptible to rust infection—increased resistance being particularly marked in the sub-group including the largest of the plants.

The seed employed was a commercial "Swedish Select" oats in which we should expect a mixture of strains as regards rate of growth, speed of maturity, and susceptibility to rust. In view of the uncertainty as to the varietal purity of the seed employed, the differences in incidence of rust infection on plants receiving the same treatment and showing differences in vigor of growth are probably indicative of constitutional differences in susceptibility to rust which may be correlated with similar constitutional differences in speed of growth; and so may be considered as not necessarily bearing on the main problem I am considering, which is concerned with the effect on rust susceptibility of externally induced variations in the vegetative vigor of the host. The establishment of an inverse relation between susceptibility to rust and speed of growth in oat varieties would, however, lend new significance to the practical injunction of the agronomists to plant early-maturing varieties of oats in order to escape loss from rust, indicating that selection of rapidly growing and early-maturing strains of oats automatically implies selection for rust resistance as well.

The Possibility of a Direct Relation between Environmental Conditions and Rust Resistance

Groups *D*, *E*, and *F* in experiments IV and V were intended as tests for a possible direct effect on rust susceptibility of specific nutrient substances—that is, an effect independent of variations in the health and vigor of the host plant. A potash fertilizer was applied to the plants of group *D*; a phosphate fertilizer to those of group *E*; and the plants of group *F* were richly fed with a nitrogen salt.

The infection observed in these groups is in no instance so far different from that on plants of similar weight in the groups not treated with any special fertilizer as to justify the inference that the fertilizing chemicals were exerting any influence on the rust resistance of the host other than is implied in their effect on the general condition and vigor of the plant.

In experiment IV the potash and phosphate applications proved excessive, and the growth of the plants was appreciably retarded as compared with the plants of group *C*; in experiment V the potash and phosphate fertilizers had no effect on the growth of the plants. In both experiments the potash- and phosphate-fertilized plants show a somewhat higher incidence of infection than plants of similar weight not treated with special fertilizers; a tendency at variance with the statements of Bolley (1889, p. 18) and Spinks (1913, p. 247) that these fertilizers give increased rust resistance. In group *F* the stimulating action of the nitrate fertilizers on the growth of the host was so marked that there can be no hesitation in referring the increased susceptibility observed to this effect rather than to any direct action of the chemical. This aspect of the soil-culture experiments may be considered as in agreement with the suggestion arrived at in the bibliographical review that it is questionable whether a direct relation between any environmental factor, either physical or chemical, of the nature of a nutrient or a stimulus, and susceptibility to rust, has been established in the case of the cereal grains.

Vegetative Vigor of the Host as a Susceptibility- and Resistance-factor in Infectious Diseases

Increased susceptibility with increased vigor of the host, in plant diseases, is not confined to the rusts. Marchal (1902) found that infection of lettuce by *Bremia lactucae* was favored by nitrogen and phosphates and retarded by an excess of potash. Jones (1905, p. 38) mentions that high fertilization, especially with nitrogenous manures, lowers the powers of the potato plant to resist blight and rot. McCue (1913, p. 18) observed that tomato plants treated with phosphatic fertilizers developed less leaf blight than control plants, while plants on nitrogen and potash plots which at the same time gave the highest yields, indicating greatest vigor of growth, were more heavily infected than the controls. Peltier (1918) has observed with the citrus canker, and Fromme and Murray (1919, p. 227) with the angular leaf spot of tobacco ("the development of the organism within the tobacco leaf is apparently dependent to a marked degree on those predisposing factors which promote a rapid, vigorous growth of the host"), that infection is heavier under conditions which favor the growth of the host. Thomas (1921) obtained evidence of increased resistance to leaf spot (*Septoria Apii*) of celery plants the vitality of which was depressed as a result of infestation of the root system by nematodes; and of decreased resistance in plants richly fed. And Levine (1921) has observed that crown gall on beets developed more rapidly and to larger size on roots grown in a highly manured soil.

While the claim that increased vigor of the host means greater susceptibility to an infection may appear somewhat anomalous from the point of view of current theories regarding the infectious diseases, observations such as form the subject of the present paper are readily understood when we consider the infectious diseases in the light of the larger class of biological phenomena of which they are an artificially selected group—namely, parasitism, commensalism, and symbiosis, the class of biological phenomena in which one organism lives within, and derives its sustenance from, the tissues of another living organism. In each of the four main groups of parasitic organisms—the bacteria, the protozoa, the worms, and the fungi—a series of intergradations are to be observed in the physiological interrelations of host and parasite, from the unceasing and violent struggle that continues until the destruction of one or other of the principals, to a relation of a more benign type characterized by great subordination and even tendency to usefulness on the part of the parasitic organism, and by the utmost tolerance on the part of the host. In many instances the nature of the reaction is not constant, but varies with the progress of the host-parasite relation. In this intergrading series of possible host-parasite relations, the inverse relation between host vigor and parasite virulence obtains only in the instances and phases where the reaction of the host to the parasite is one of active antagonism; here a more vigorous host means a host of greater physiological capacity to combat the progress of the invader. But when the relation between host and parasite is of a symbiotic type, a more vigorous host means a host in which more food is available for the development of the parasite. Because, of the general class of parasitological phenomena, the instances mainly in the field of pathological interest (the diseases ordinarily so called) are an artificially selected group in which relations of violent antagonism between host and invading organism are most prominently in evidence, thought in the field of pathology has developed with the physiological antagonism of host and parasite as its basal concept; and the theories of immunity extant are largely concerned with the nature of the antagonistic reactions.

In the group of the fungi the transition from violent and destructive parasitism to parasitism of the symbiotic type is accompanied by a transition from facultative to obligate parasitism, as if the physiological corollary of parasitism of the latter type is extreme specialization in food preferences. The series in the fungi grades from violent and destructive parasites like Botrytis, on the one hand, to, on the other hand, so benign an infestation as the seed fungus of *Lolium temulentum* (described by Freeman, 1903) in which the relation is so intimate and devoid of any untoward effect on the host, and the life history of the cohabiting organism is so parallel with that of the grass, that its distinct individuality is almost open to question.

The mutualistic nature of the relation between host elements and fungus in rusts of the type of the cereal rusts is commented on by Tubeuf (1897, p. 91) who very aptly compares the mass of chlorophyll-bearing leaf cells infested with the rust mycelium to a lichen structure, especially to those lichens whose algae obtain water and inorganic materials direct, rather than through the fungous hyphae. Certainly, during the greater part of the relation, there is here no evidence of any deleterious effects on the host cells. While the contribution of the affected elements to the growth and fruiting economy of the host plant as a whole may be diminished, the infected protoplasts continue essentially unimpaired in structure and function. The parasite does not attack the living substance of the host protoplast, but confines itself to establishing such a relation with the latter that it shares the available food resources of the cell; and the rust haustorium is not an implement for mechanical disruption, but a structure more in the nature of the placenta of the mammalian foetus for establishing physiological communication with the food resources of the host.

The data presented by Thomas (1921) on the parallel relation between health of the host and infection in the case of the leaf blight of celery, and observations of similar occurrences in other diseases caused by non-obligate parasites like the late blight of the potato (Jones, 1905) and the crown gall of the beet (Levine, 1921) indicate that phases in which a symbiotic tendency comes to the fore may occur in diseases of a predominantly destructive type caused by facultative parasites, and suggests the generalization that the host-parasite relation in any given instance is not constant but may vary with the state and condition of the organisms and with the progress of the relation. It is important to recognize that there may occur mutualistic phases and stages in host-parasite relations of a violent and destructive type, just as there are destructive phases in parasitisms of a predominantly symbiotic tendency such as those of the mildews, the rusts, and the smuts.

Conclusion

The inquiry initiated by the occurrence in rust literature of statements of a relation between host vigor and susceptibility other than the inverse relation commonly conceived as existing between these variables can be considered as having brought forward evidence indicating that through most of the course of certain infectious diseases such as the rust diseases of the cereal grains, and in certain phases of other diseases like the leaf spot of celery and the crown gall of the beet, the vegetative vigor of the host and the virulence of the disease may be in direct relation. The demonstration of such a relation in diseases of large importance suggests, in turn, emendation of current pathological concepts of the relation between host vigor and pathogen activity into a form more in accord with our knowledge of parasitological phenomena in general. A more catholic point of view in pathologic thought, recognizing that, for longer or shorter phases in the course of a disease, the relation between host and parasite may be highly mutualistic, would be of material value as a working concept in the study of disease and in defining the practical problem of disease prevention and control.

The work presented in this paper was done in the Botanical Laboratory of Columbia University, under Professor R. A. Harper, to whom the writer is greatly indebted for pointing out the problem and for constant consultation and advice during the progress of the investigations. Acknowledgment is also made of indebtedness to Dr. Michael Levine for taking the photographs of the soil-culture experiments, and to Dr. H. E. Thomas for helpful advice in devising the method used in the dosage studies on the corn rust.

LITERATURE CITED

Adami, J. G. The principles of pathology. Vol. 1. Philadelphia and New York, 1910.

Arthur, J. C. Problems in the study of plant rusts. Bull. Torr. Bot. Club **30**: 1–18. 1903.

Bailey, L. H. Cyclopedia of horticulture. New York, 1914.

Bailey, M. A. *Puccinia malvacearum* and the mycoplasm theory. Annals of Bot. **34**: 73–100. 1920.

Biffen, R. A. Studies in the inheritance of disease resistance. Jour. Agr. Sci. **4**: 421–429. 1912.

Bolley, H. L. Wheat rust. Ind. Agr. Exp. Sta. Bull. 26. 1889.

Burnham, S. H., and Latham, R. A. The flora of the town of Sothold, Long Island, and Gardiner's Island. Torreya **14**: 201–225, 229–254. 1914.

Butler, E. J. Fungi and disease in plants. Calcutta, 1918.

——**, and Hayman, J. M.** Indian wheat rusts. Mem. Dept. Agr. India, Bot. Ser. **12**: 1–58. 1906.

Christman, A. H. Observations on the wintering of grain rusts. Trans. Wis. Acad. Sci., Arts, and Lett. **15**: 98–107. 1905.

Clinton, G. P. Report of the Connecticut State Agricultural Experiment Station. 1903.

Eriksson, J., and Henning, E. Die Getreideroste. Stockholm, 1896.

——**, and Hammarlund, C.** Essais d'immunisation de la Rose tremière contre la maladie de la Rouille. Compt. Rend. Acad. Sci. Paris **158**: 420–422. 1914.

Fischer, E. Die Uredineen der Schweiz. Bern, 1904.

Freeman, E. M. The seed fungus of *Lolium temulentum* L., the darnel. Phil. Trans. Roy. Soc. Lond. B **196**: 1–27. Pls. 1–3. 1903.

——**, and Johnson, E. C.** The rusts of the grains in the United States. U. S. Dept. Agr. Bur. Plant Ind. Bull. 216. Pp. 87, 2 fig., 1 pl. 1911.

Fromme, F. D. The culture of the cereal rusts in the greenhouse. Bull. Torrey Bot. Club **40**: 501–521. 1913.

——**, and Murray, T. J.** Angular leaf-spot of tobacco, an undescribed bacterial disease. Jour. Agr. Res. **16**: 219–228. 1919.

Galloway, B. T. Experiments in the treatment of rusts affecting wheat and other cereals. Jour. Mycol. **7**: 195–226. 1893.

Gassner, G. Die Teleutosporenbildung der Getreiderostpilze, und ihre Bedingungen. Zeitschr. Bot. **7**: 65–120. 1915.

Gibson, C. M. Notes on infection experiments with various Uredineae. New Phytol. **3**: 184–191. 1904.

Giddings, N. J. Infection and immunity in apple rust. W. Va. Agr. Exp. Sta. Bull. 170. 1918.

Grove, W. B. The British rust fungi. Cambridge, 1913.

Hecke, L. Zur Frage der Überwinterung des Gelbrostes und das Zustandekommen von Rostjahren. Naturwiss. Zeitschr. Forst- u. Landw. **13**: 213–220. 1915.

Humphrey, J. E. Report of the Massachusetts State Agricultural Experiment Station. 1891.

Hungerford, C. W. Rust in seed wheat and its relation to seedling infection. Jour. Agr. Res. **19**: 257–277. 1920.

Jelliffe, S. E. The flora of Long Island. Lancaster, Pa., 1899.

Johnson, E. G. Cardinal temperatures for the germination of uredospores of cereal rusts (Abstr.). Phytopath. **2**: 47. 1912.

Jones, L. R. Disease resistance of potatoes. U. S. Dept. Agr. Bur. Plant Ind. Bull. 87. 1905.

Jost, L. Lectures in plant physiology (Eng. transl. by Gibson). Oxford, 1907.

Kolmer, J. A. A practical text-book of infection, immunity, and specific therapy. Philadelphia, 1917.

Laurent, E. Recherches expérimentales sur la mal des plantes. Ann. Inst. Past. **13**: 1–48. 1899.

Lauritzen, J. I. The relation of temperature and humidity to infection by certain fungi. Phytopath. **9**: 7–36. 1919.

Levine, M. Studies on plant cancers III. The nature of the soil as a determining factor in the health of the beet, *Beta vulgaris*, and its relation to the size and weight of the crown gall produced by inoculation with *Bacterium tumefaciens*. Amer. Jour. Bot. **8**: 507–525. 1921.

Little, W. C. Report on wheat mildew. Jour. Roy. Agr. Soc. Eng. **19**: 634–693. 1883.

McAlpine, D. The rusts of Australia. Melbourne, 1906.

McCue, C. A. Tomatoes for the canning factory. Del. Agr. Exp. Sta. Bull. **101**: 1–86. 1913.

Mains, E. B. The relation of some rusts to the physiology of their hosts. Amer. Jour. Bot. **4**: 179–220. 1917.

Marchal, É. De l'immunization de la laitue contre le meunier. Compt. Rend. Acad. Sci. Paris **135**: 1067, 1068. 1902.

Melhus, I. E. Culturing of parasitic fungi on the living host. Phytopath. **2**: 197–203. 1912.

Peacock, R. W. Rust in wheat and oats. Agr. Gaz. N. S. Wales **22**: 1013–1016. 1911.

Peck, C. H. Report of the New York State Botanist. 1871.

Peltier, G. L. Susceptibility and resistance to citrus-canker of the wild relatives, citrus fruits, and hybrids of the genus Citrus. Jour. Agr. Res. **14**: 337–358. 1918.

Plowright, C. B. The British Uredineae and Ustilagineae. London, 1889.

Sheldon, J. L. Preliminary studies on the rusts of the asparagus and carnation. Science, n. ser. **16**: 235–237. 1902.

——. Infection and parasitism in Uredineae. (Typewritten thesis deposited in the library of the University of Nebraska.) Pp. 145. 1903.

——. The effect of different soils on the development of the carnation rust. Bot. Gaz. **40**: 225–229. 1905.

Smith, R. E. The water-relation of *Puccinia asparagi*. Bot. Gaz. **38**: 19–43. 1904.

——. Asparagus and asparagus rust in California. Cal. Agr. Exp. Sta. Bull. 165. 1905.

Spinks, G. T. Factors affecting susceptibility to disease in plants. Part I. Jour. Agr. Sci. **5**: 231–247. 1913.

Stakman, E. C. A study in cereal rusts; physiological races. Minn. Agr. Exp. Sta. Bull. **138**: 1–56. 1914.

——**, and Piemeisel, F. J.** Biologic forms of *Puccinia graminis* on cereals and grasses. Jour. Agr. Res. **10**: 429–496. 1917.

——**, and Levine, M. N.** Effect of certain ecological factors on the morphology of the urediniospores of *Puccinia graminis*. Jour. Agr. Res. **16**: 43–77. 1919.

Thaxter, R. Report of the Connecticut Agricultural Experiment Station, 1890.

Thomas, H. E. The relation of the health of the host and other factors to infection of *Apium graveolens* by *Septoria apii*. Bull. Torrey Bot. Club **48**: 1–29. 1921.

Tubeuf, K. F. von. Diseases of plants (Eng. transl. by Smith). London, 1897.

Voelcker, J. A. The Woburn pot-culture experiments, 1910–1911–1912. Jour. Roy. Agr. Soc. Eng. **73**: 314–338. 1912.

Ward, H. M. On pure cultures of a Uredine, *Puccinia dispersa* (Erikss.). Proc. Roy. Soc. London **69**: 451–466. 1902 (*a*).

——. Experiments on the effect of mineral starvation on the parasitism of the Uredine fungus, *Puccinia dispersa*, on species of Bromus. Proc. Roy. Soc. London **71**: 138–151. 1902 (*b*).

——. On the relations between host and parasite in the bromes and their brown rust, *Puccinia dispersa* (Erikss.). Ann. Bot. **16**: 233–315. 1902 (*c*).

——. Recent researches on the parasitism of fungi. Ann. Bot. **19**: 1–55. 1905.

Wilson, J. K. Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. **2**: 420–428. 1915.

Zavitz, C. A. Thickness of seeding in cereal grains. Ann. Rep. Canadian Seed Growers' Assoc. **9**: 39–45. 1913.

Zinsser, H. Infection and resistance. New York, 1914.

DESCRIPTION OF PLATES

PLATE XI

FIG. 1. View of one of the four series of pots of experiment V of the soil-culture studies, illustrating the difference in vigor of growth between plants receiving different nutritive treatment.

FIG. 2. On the left, a pot containing 5 plants of group *A* (grown in sand); and on the right a pot containing 5 plants of group *F* (soil highly fertilized with $NaNO_3$); experiment V of the soil-culture studies.

PLATE XII

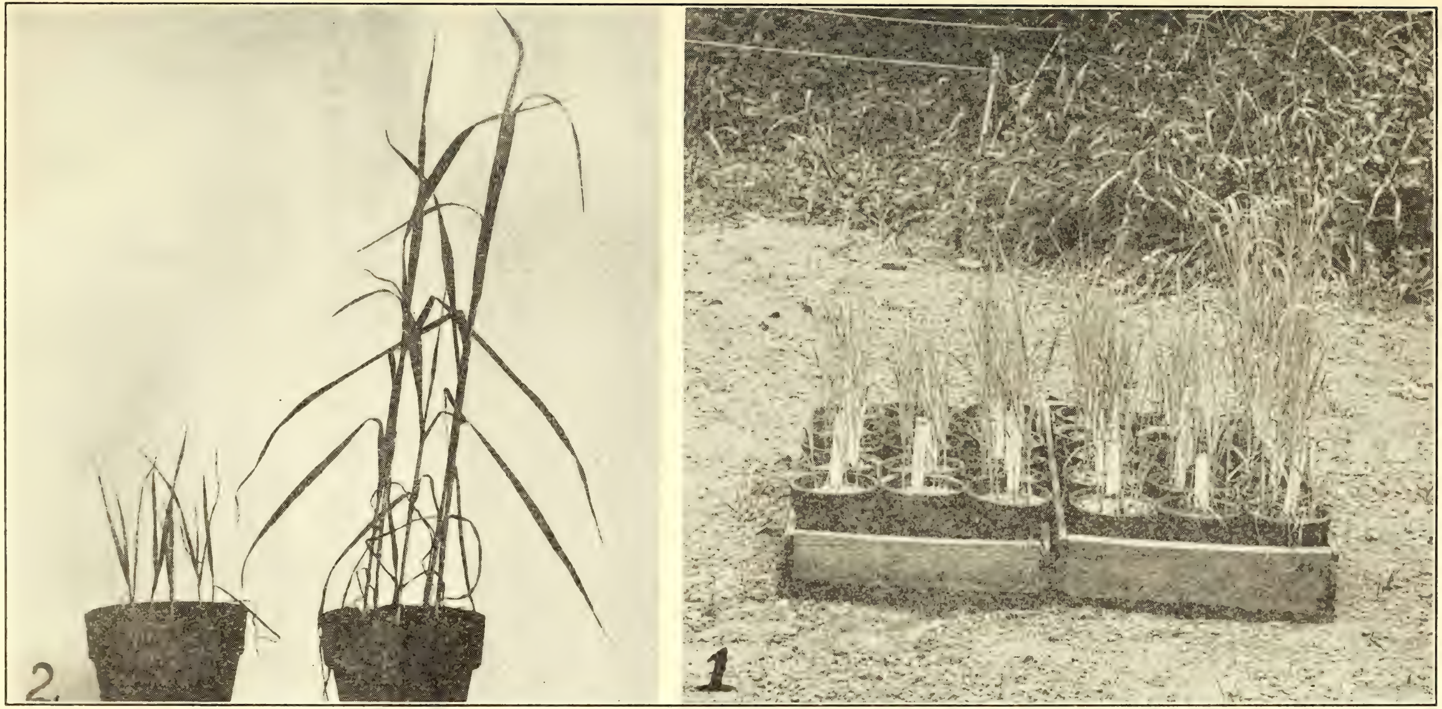
FIG. 3. Crown rust of oats. A rust mycelium exhibiting a very marked tendency towards teleutospore production. The first sorus produced by the mycelium (in the center) is a uredosorus. The others are teleutosori. Photographed with Zeiss 3.5 cm. microplanar. × 24.

FIG. 4. View of infected leaves of a semi-starved plant and of a richly fed plant of soil-culture experiment V, showing larger size of pustules on more luxuriant host plant. Photographed with Zeiss microplanar. × 15.

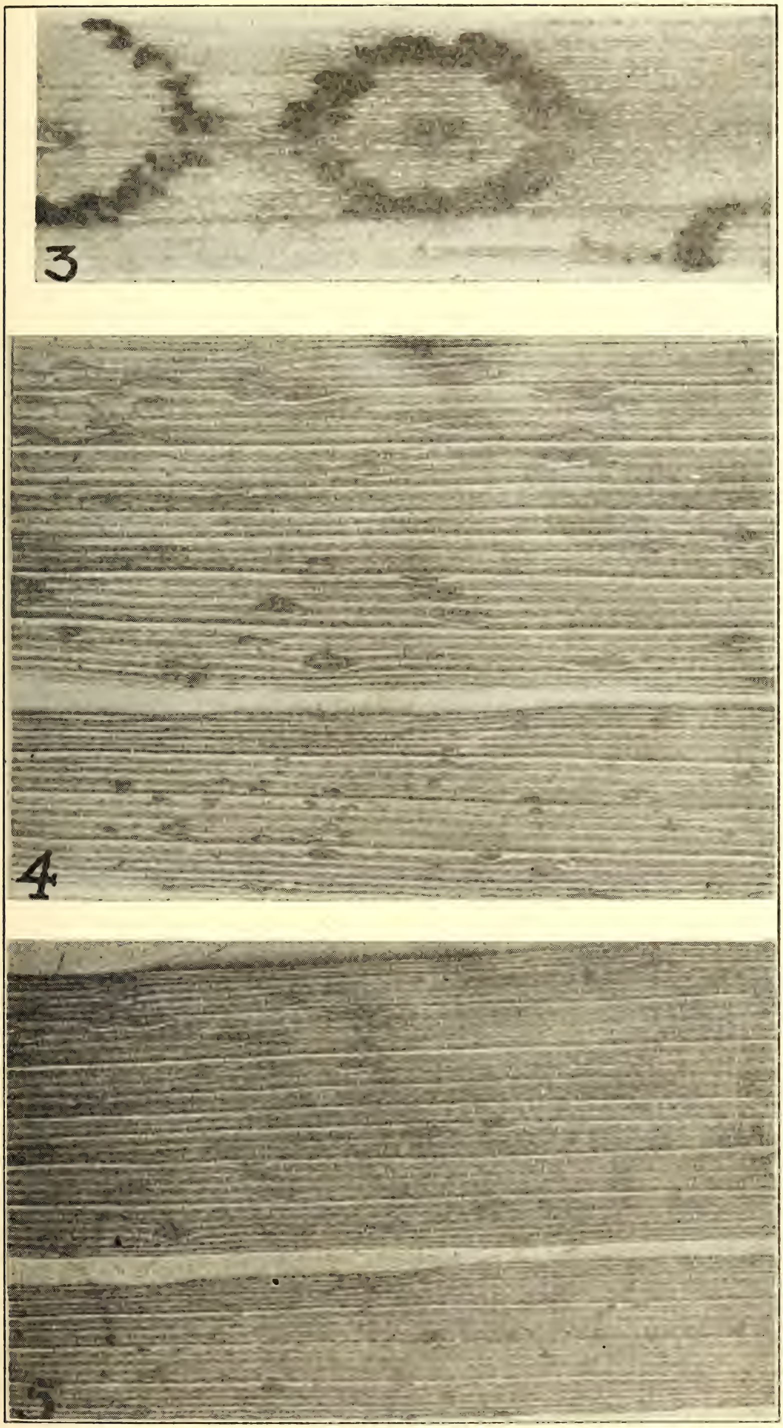
FIG. 5. Same as figure 4. × 10.

ERRATUM

In the first part of this paper, appearing in the **Journal** for April, 1922, pp. 183–203, on page 188, line 32, and on page 193, line 1, for *Puccinia Caryophylli* read *Uromyces caryophyllinus.*



RAINES: VEGETATIVE VIGOR OF THE HOST



Raines: Vegetative Vigor of the Host

LIGNIFICATION OF MATURE PHLOEM IN HERBACEOUS TYPES

Carl L. Wilson

(Received for publication August 5, 1921)

Secondary growth in the woody stems of gymnosperms and angiosperms often results in the formation of annual rings in the xylem, but in the phloem no similar arrangement of elements is usually to be observed. The sieve tubes, which are generally the principal elements of the phloem, are crushed at the end of each period of growth by the pressure of surrounding cells and tissues. To these changes taking place in the secondary phloem the term *obliteration* has been applied for a long time. The alterations are initiated by the disappearance of the contents of the sieve tubes at the end of each growth period, soon followed by a crushing and distortion of the sieve tubes, and, in the case of angiosperms, of companion cells also. The sieve plates may be broken down, and the wall of the sieve tube takes on a swollen appearance. In an advanced condition of obliteration, the lumen of the sieve tube entirely disappears, being represented only by a line, and the whole mass of crushed sieve tubes takes on a horn-like consistency. During these changes the structure of the phloem parenchyma does not appear to be affected.

The term horn prosenchym (*Horngewebe*) was applied by Wigand (1863) to such a mass of crushed tissue. The principal facts concerning the transformations undergone by the phloem of woody angiosperms have been generally known since the time of this writer. Previous to this date both Oudemans (1862) and Rauwenhoff (1869) appear to have described obliterated structures in the phloem, Oudemans in 1855, and Rauwenhoff in 1859. These two writers, together with Moeller (1875) and Tschirch (1889), showed the crushed tissue to be composed of obliterated sieve tubes. Tschirch pointed out that the sieve tubes, including cambiform cells and companion cells, having lost the principal portion of their contents at the end of the growing season, become pressed together through the turgor of neighboring cells, so that only a small, cleft-shaped lumen remains. He believed that a subsequent thickening of the wall does not take place. De Bary (1884) indicated some doubt as to the extent to which the cambiform cells of the phloem are affected in obliteration, although he described the crushing of the sieve tubes. Bliesnick (1891) found that the phloem parenchyma takes no part in obliteration. His conclusions are essentially the same as those of Tschirch with regard to the causes of obliteration. Lignification of sieve plates was found in Clematis, and in another case he mentions that he found complete lignification of the obliterated phloem elements. His results differ from those of previous investigators in that he found sieve tubes and companion cells crushed, but never phloem parenchyma.

Publications on the changes undergone by the phloem of herbaceous types are few. Schumann (1890), in his work on the anatomy of the composite stem, reports finding lignification of the sieve tubes and companion cells in two species of Compositæ, *Scorzonera Hispanica* and *Aster thyrsiflorus*, finding the sieve plates especially to be lignified. Boodle (1902), in his first note on the subject, reports finding lignification of the sieve tubes and companion cells in two plants of *Helianthus annuus;* lignification of contents, though not of the walls of sieve tubes, was obtained in the root. In repeating the work of Vesque and Boubier, Boodle found no lignification of sieve tubes in *Betula alba*, such as had been described by these workers. In a later publication, Boodle (1906) reported results of the examination of additional material of Helianthus, in the course of which examination he investigated eleven plants of *H. annuus*, one plant of *H. tuberosus* L., and the stems of *H. laetiflorus* Pers. and *H. decapetalus* L. The observations previously recorded were confirmed both for *H. annuus* and for the other species mentioned. His conclusions may be summed up as follows: the sieve tubes and companion cells in the stems of the species mentioned are normally lignified; the contents of the sieve tubes in the root become lignified, whereas lignification of phloem parenchyma is rare, having been observed in only one case.

The present investigation was undertaken to determine whether there exist any departures from the usual unlignified and uncrushed condition of the phloem in herbaceous types, without reference to certain alterations known to be brought about by diseased conditions, such as phloem necrosis in leafroll of potatoes.

In order to determine the occurrence of such unusual conditions, the mature stems of a large number of herbaceous plants were examined by means of free-hand sections.[1] No conditions departing from the usual were found, until species of the Compositae were examined. In a number of species of this family extensive lignification of the phloem was found to have taken place. This lignification would seem to include only the parenchyma of the phloem, other elements presenting the usual appearance of a phloem group of the herbaceous type. The lignified elements appear with greatly thickened walls, and, but for their position, would in most cases be difficult to distinguish from the tracheids of the xylem. In a phloem group in which such lignification is found, it is practically certain that all of the phloem parenchyma becomes lignified, with the exception of those elements most recently formed, found next to the cambium. The parenchyma cells of this region, upon reaching maturity, will of course also become lignified. No conditions such as were described by Schumann and Boodle have been noted. The sieve tubes and companion cells have not been found to be lignified, and appear normal and functional in every way. In the transverse section of a stem shown in figure 1, Plate XIII, (upper left), a phloem group is seen enclosed between the bundle cap and the xylem. All the elements exclusive of the sieve tubes and companion cells appear lignified with the exception of several groups just outside the cambium; these latter may be tissue which has not yet matured, or may consist of sieve tubes. In the transverse section shown in figure 2 (upper right), the whole of the phloem is not shown, but the same condition is seen to prvail. Some unlignified elements are seen just under the bundle cap; these are probably sieve tubes of the protophloem. Figure 3 (lower left) is an enlarged view of a portion of the same region shown in figure 2, showing unlignified sieve tubes and companion cells surrounded by lignified parenchyma. Figure 4 (lower right) represents a longitudinal section through a region similar to that shown in the preceding figure. The walls of the sieve tubes (*s*) appear black, in contrast to the lighter color of the lignified parenchyma walls. Several cambiform cells with lignified walls are indicated at *x*, a nucleus and stored food material being clearly shown.

---

[1] The sections were stained on the slide with phloroglucin and concentrated hydrochloric acid, the lignified portions giving the characteristic reaction. Portions of stems showing typical lignification were then imbedded in celloidin, sectioned, and stained with haematoxylin and safranin.

Some correlation, at least, appears to exist between the presence of lignification in the stem and in the root. In an examination of the tap roots of several species possessing lignification, lignification of phloem parenchyma was found. The following plants were found to possess lignified phloem elements in both root and stem:

Solidago arguta Ait.
S. rugosa Mill.
S. serotina Ait.
Aster ericoides L.
A. umbellatus Mill.
A. puniceus L.
Solidago graminifolia (L.) Salisb.

As stated previously, no conditions comparable to those described by Boodle have been noted, either in *Helianthus annuus* or in other plants to which he referred. An examination of a large number of stems of *H. annuus* was made to find such conditions if possible. In one case only was a departure from the usual condition found. This was in a mature stem, about one half inch in diameter, which exhibited an unmistakable lignification of phloem parenchyma when examined by means of free-hand and microtome sections.

Referring to the following list, several interesting facts may be brought out. It will be noted that all the forms in which lignification has been found belong to the first series of the Compositae, the Tubuliflorae, no lignification having been found in any species falling within the Liguliflorae. Lignification does not seem to occur in all the species of a genus in which lignification has been found. Illustrations of this fact can be seen in nearly all the genera listed. It has also been found in a few cases, which could probably be extended by further observation, that lignification is not found in every individual of the same species.

The following material has been studied in the course of the investigation:

Species of Compositae Having Lignified Phloem Elements

Grindelia squarrosa (Pursh) Dunal
Solidago latifolia L.
S. bicolor L.
S. puberula Nutt.
S. sempervirens L.
S. patula Muhl.
S. arguta Ait.
S. juncea Ait.
S. uniligulata (DC.) Porter
S. ulmifolia Muhl.
S. rugosa Mill.
S. nemoralis Ait.
S. canadensis L.
S. altissima L.
S. serotina Ait.
S. graminifolia (L.) Salisb.
Aster divaricatus L.
A. Schreberi Nees
A. macrophyllus L.
A. novae-angliae L.
A. patens Ait.
A. undulatus L.
A. cordifolius L.
A. Lowrieanus Porter
A. sagittifolius Wedemeyer
A. laevis L.
A. ericoides L. var. *villosus* T. & G.
A. multiflorus Ait.
Aster dumosus L.
A. vimineus Lam.
A. lateriflorus (L.) Britton
A. Tradescanti L.
A. paniculatus Lam.
A. junceus Ait.
A. prenanthoides Muhl.
A. puniceus L.
A. umbellatus Mill.
A. subulatus Michx.
A. angustus (Lindl.) T. & G.
Erigeron annuus (L.) Pers.
E. ramosus (Walt.) BSP.
E. canadensis L.
Sericocarpus asteroides (L.) BSP.
Anaphalis margaritacea (L.) B. & H.
Gnaphalium polycephalum Michx.
G. decurrens Ives
Helianthus annuus L.
Coreopsis tripteris L.
Achillea Ptarmica L.
A. Millefolium L.
Anthemis Cotula L.
Tanacetum vulgare L.
Artemisia caudata Michx.
A. Pontica L.
A. Absinthium L.

Species of Compositae Examined and Found to Contain No Lignified Phloem Elements

Vernonia noveboracensis Willd.
V. altissima Nutt.
Eupatorium purpureum L.
E. hyssopifolium L.
E. sessilifolium L.
E. urticaefolium Reichard
Mikania scandens (L.) Willd.
Liatris scariosa Willd.
Solidago caesia L.
Erigeron pulchellus Michx.
Antennaria plantaginifolia (L.) Richards.
A. fallax Greene
A. neodioica Greene
A. petaloidea Fernald
Gnaphalium uliginosum L.
Inula Helenium L.
Polymnia canadensis L.
Silphium trifoliatum L.
Ambrosia trifida L.
A. artemisiifolia L.
Xanthium canadense Mill.
X. commune Britton
X. echinatum Murr.
Heliopsis helianthoides (L.) Sweet
Rudbeckia hirta L.
R. laciniata L.
Helianthus divaricatus L.
H. strumosus L.
H. decapetalus L.
H. tuberosus L.
Bidens discoidea (T. & G.) Britton
B. frondosa L.

Bidens vulgata Greene
B. comosa (Gray) Wiegand
B. cernua L.
B. Beckii Torr.
Galinsoga parviflora Cav.
Helenium autumnale L.
Anthemis tinctoria L.
Chrysanthemum Leucanthemum L. var. *pinnatifidum* Lecoq & Lamotte
C. Parthenium (L.) Bernh.
C. Balsamita L. var. *tanacetoides* Boiss.
Artemisia biennis Willd.
Tussilago Farfara L.
Erechtites hieracifolia (L.) Raf.
Senecio vulgaris L.
Cacalia atriplicifolia L.
Arctium Lappa L.
A. nemorosum Lejeune
Cirsium muticum Michx.
Cirsium pumilum (Nutt.) Spreng.
C. arvense (L.) Scop.
Centaurea Cyanus L.
C. nigra L.
Lapsana communis L.
Cichorium Intybus L.
Krigia virginica (L.) Willd.
Leontodon autumnalis L.
Tragopogon pratensis L.
Chondrilla juncea L.
Sonchus oleraceus L.
S. asper (L.) Hill
Lactuca scariola L.
Prenanthes serpentaria Pursh
P. trifoliolata (Cass.) Fernald
Hieracium aurantiacum L.
H. venosum L.
H. scabrum Michx.

SPECIES OF OTHER FAMILIES IN WHICH LIGNIFICATION HAS NOT BEEN FOUND

Saururus cernuus L.
Cannabis sativa L.
Rumex crispus L.
Polygonum Hydropiper L.
P. acre HBK.
P. virginianum L.
P. scandens L.
Atriplex patula L. var. *hastata* (L.) Gray
Amaranthus retroflexus L.
A. blitoides Wats.
Phytolacca decandra L.
Mollugo verticillata L.
Lychnis alba Mill.
Silene sp.
Saponaria officinalis L.
Ranunculus acris L.
Thalictrum dioicum L.
T. polygamum Muhl.
Anemone virginiana L.
Menispermum canadense L.
Sisymbrium officinale (L.) Scop.
Sedum purpureum Tausch
Potentilla monspeliensis L.
P. recta L.
Agrimonia gryposepala Wallr.
A. striata Michx.
Melilotus alba Desr.
Medicago sativa L.
Vicia villosa Roth
Lathyrus palustris L.
Amphicarpa monoica (L.) Ell.
Linum usitatissimum L.
Oxalis corniculata L.
Acalypha virginica L.
Althaea rosea Cav.
Lythrum Salicaria L.
Rhexia sp.
Oenothera biennis L.
Sanicula marilandica L.
Pastinaca sativa L.
Apocynum sp.
Asclepias incarnata L.
A. syriaca L.
Convolvulus sepium L.
Cuscuta Gronovii Willd.
Myosotis scorpioides L.
Verbena urticaefolia L.
Nepeta Cataria L.
Leonurus Cardiaca L.
Monarda didyma L.
Collinsonia canadensis L.
Solanum Dulcamara L.
Datura Tatula L.
Verbascum Blattaria L.
Linaria vulgaris Hill
Scrophularia leporella Bicknell
Plantago major L.
Dipsacus sylvestris Huds.
Cucurbita sativus L.
Echinocystis lobata (Michx.) T. & G.
Lobelia cardinalis L.
L. siphilitica L.

SUMMARY

It has been commonly supposed that no crushing, or alterations involving a change in the chemical composition of the cell wall, occurs in the mature phloem of herbaceous angiosperms, although extensive alterations are known to take place in the mature woody stems of gymnosperms and angiosperms. It has been found, however, that within the series Tubuliflorae of the family Compositae, extensive lignification of the phloem parenchyma takes place in a number of species belonging to several different genera. These elements appear to retain their functional activity. The structure of the sieve tubes and companion cells is not affected.

The writer wishes to acknowledge the advice and assistance received during the course of the investigation from Dr. L. H. MacDaniels, of the Department of Pomology, New York State College of Agriculture, and from Dr. Ernst Artschwager, United States Bureau of Plant Industry, Washington, D. C.

DEPARTMENT OF BOTANY,
NEW YORK STATE COLLEGE OF AGRICULTURE,
CORNELL UNIVERSITY

BIBLIOGRAPHY

de Bary, A. Comparative anatomy of the vegetative organs of the phanerogams and ferns English translation by Bower and Scott, p. 542. Oxford, 1884.

Bliesnick, H. Über die Obliteration der Siebröhren (Thesis). Berlin, 1891.

Boodle, L. A. On lignification in the phloem of *Helianthus annuus.* Annals of Bot. **16**: 180–183. 1902.

————. Lignification of phloem in Helianthus. Annals of Bot. **20**: 319–321. 1906.

Moeller, J. Über die Entstehung des Akacien-Gummi. Sitzungsber. K. Akad. Wiss. Wien Math.-Naturw. Kl. **72**^{2}: 219–230. 1875.

Oudemans, C. A. J. A. Das Hornprosenchym Wigand's. Bot. Zeit. **20**: 43–44. 1862.

Rauwenhoff, N. W. P. Observations sur les caractères et la formation du liége dans les Dicotylédones. Ann. Sci. Nat. Bot. V, **12**: 347–364. 1869.

Schumann. Beitrag zur Anatomie des Compositenstengels. Bot. Centralbl. **41**: 193–196. 1890.

Tschirch, A. Angewandte Pflanzenanatomie, pp. 342–352. Vienna and Leipsic, 1889.

Wigand, A. Ueber die Deorganisation der Pflanzenzelle. Jahrb. Wiss. Bot. **3**: 115–182. 1863.

EXPLANATION OF PLATE XIII

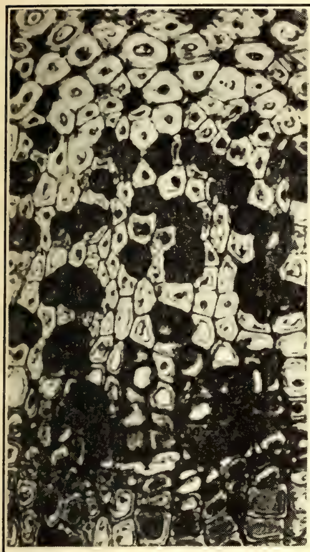
FIG. 1. Transverse section of phloem group from the stem of *Gnaphalium polycephalum.* Note contrast between lignified parenchyma and darkly stained, unlignified sieve tubes.

FIG. 2. Transverse section, stem of *Aster paniculatus,* showing portions of the bundle cap, protophloem, and secondary phloem.

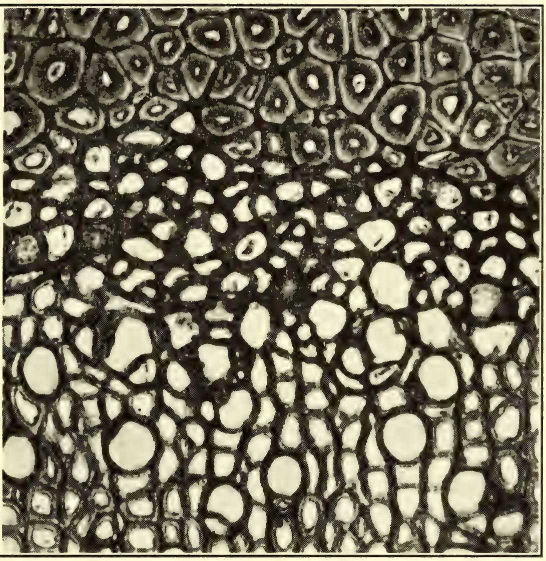
FIG. 3. Enlarged view of a portion of the phloem group shown in figure 2.

FIG. 4. Longitudinal section of the phloem of the stem of *Aster novae-angliae,* showing unlignified sieve tubes (*s*) and lignified parenchyma (*x*).

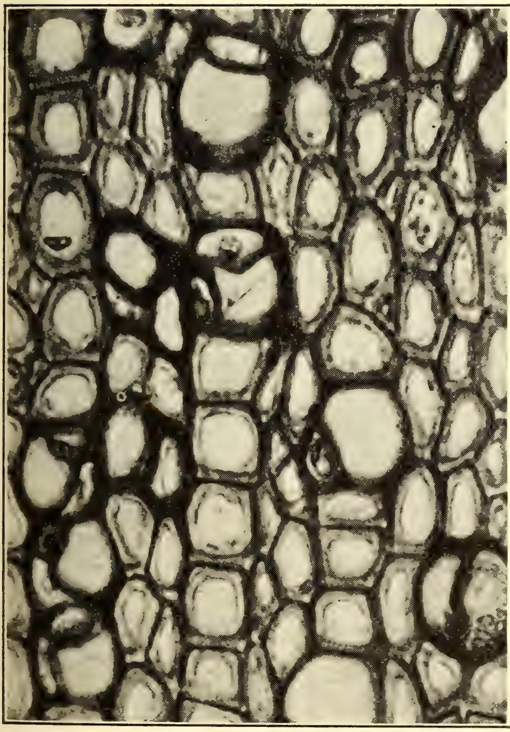
1



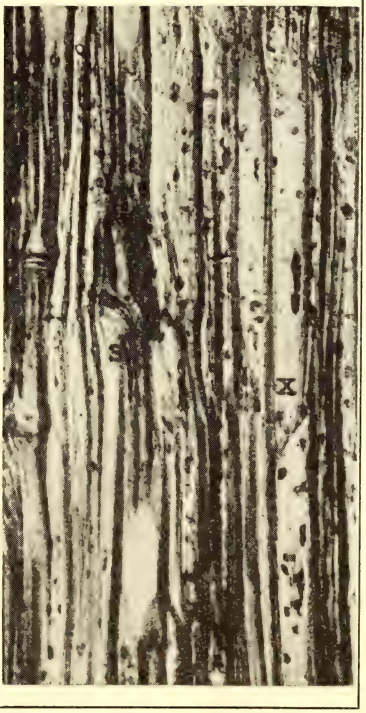
2



3



4



WILSON: LIGNIFICATION OF MATURE PHLOEM

OBSERVATIONS ON THE EFFECT OF WATER-RAKING ON THE KEEPING QUALITY OF CRANBERRIES

H. F. Bergman[1]

(Received for publication August 11, 1921)

The practice of "water-raking" cranberries—that is, of flooding the bog and "raking" or "scooping" up the berries as they float on or near the surface of the water—is prevalent among many of the Wisconsin cranberry growers. This practice has raised the question of the effect of the more or less prolonged submergence on the keeping quality of the berries. It is often asserted that "water-raked" cranberries do not keep well. Many growers believe that even the flooding of a bog to prevent frost injury to the ripening fruit has a detrimental effect on their keeping quality. On the other hand, it is claimed by others that water-raked berries keep as well as dry-raked if properly handled.

No previous work on water-raking in relation to the keeping quality of cranberries has been done. Accordingly a series of observations was made to secure evidence as to the occurrence of injury which may be properly attributed to water-raking, the causes of injury, the conditions under which it may occur, and how it may be eliminated or reduced.

The work was done in Wisconsin during the cranberry-picking seasons of the years 1918 and 1919. In 1918 field observations were followed by counting tests of berries in storage at Chicago, Ill., and Minneapolis, Minn.

Observations on the Keeping Quality of Dry- and Water-raked Cranberries in Storage

In attempting to secure some indication as to the effect of water-raking on the keeping quality of cranberries in storage, a number of counting tests have been made on berries from various sources. In these tests as many data as possible have been secured as to the previous treatment of the berries which might affect their keeping quality. Such tests, naturally, do not have the value of experimental results, since the lots of berries from which these counts have been made represent different growing conditions and different treatments during harvest and subsequently until their arrival at the warehouse where the counts were made. However, the figures are of some value as "indicators." In some instances in which lots of dry- and water-raked berries have been taken from the same marsh and have been kept under essentially the same conditions since harvest, the results are nearly as valuable as if obtained from definitely planned experiments. Some figures obtained from counts on miscellaneous lots are given in table 1.

---

[1] Formerly Assistant Pathologist, Bureau of Plant Industry, Washington, D. C.

TABLE 1. *The Percentage of Sound and Soft Berries in Miscellaneous Lots of Cranberries in Storage at Chicago during the Autumn of 1918*

Variety	Date Examined	Method of Picking	Total No. Counted	Sound		Soft	
				Number	Percentage	Number	Percentage
Metallic Bell......	Oct. 18	Water-raked	705	672	95.3	33	4.7
Metallic Bell......	“ 18	“ “	494	428	86.7	66	13.3
Metallic Bell......	“ 18	“ “	603	524	86.9	79	13.1
Bennet Jumbo....	Nov. 23	“ “	622	556	89.4	66	10.6
Prolific...........	“ 23	Dry-raked	436	369	82.6	62	14.2
Prolific...........	“ 25	“ “	539	455	84.4	84	15.6
Juneau...........	“ 25	“ “	681	592	86.9	76	11.2
Metallic Bell......	“ 25	Water-raked	495	406	82.0	89	18.0
Metallic Bell......	“ 25	“ “	646	507	78.5	139	21.5
Bennet Jumbo....	“ 25	“ “	520	439	84.4	81	15.6
Bennet Jumbo....	“ 26	Dry-raked	455	406	89.2	49	10.8
Bennet Jumbo....	“ 26	“ “	391	351	89.8	40	10.2
Juneau...........	“ 26	“ “	717	579	80.8	138	19.2
McFarlin's(?).....	“ 26	“ “	714	537	75.2	177	24.8

On examination of the figures in table 1, it is evident that no greater difference is to be found between the keeping quality of dry- and water-raked berries than may be found between certain lots picked by the same method. This is to be expected, as the various lots are not comparable. Differences in the conditions under which the berries were grown, in methods of drying, varying weather conditions at different times during the drying period, and differences in methods of handling or in conditions of storage before shipment might make as great a difference in keeping quality as would be found between water- and dry-raked berries grown and handled under otherwise similar conditions.

It is apparent for the reasons indicated that conclusions as to the effect of water-raking on the keeping quality of cranberries are not warranted unless comparison is made between dry- and water-raked berries of the same variety from the same bog handled and stored under as nearly identical conditions as is possible. Such a comparison is made in table 2.

These berries were examined within a week or ten days after their arrival in Chicago, so that the total time that had elapsed since they were put in barrels was probably not more than three weeks. One sample from a barrel was taken in each case. The results here indicate clearly a superiority in the keeping quality of the dry-raked berries. The average loss for the latter is 8.4 percent, while for the water-raked berries it is 16.5 percent. The length of time that the berries were kept in water is not definitely known in all cases. The Early Blacks were flooded at night and raked the next day. Unfortunately none of these were dry-raked, so that no comparison can be made in their case except to note that they apparently have been injured somewhat less than the water-raked Metallic Bell. Some of the latter were held in water two days before raking. As counts on two barrels showed 18.1 and 21.1 percent of soft berries, it is probable that these were some which had been under prolonged flooding.

TABLE 2. *A Comparison of the Keeping Quality of Dry-raked and Water-raked Cranberries in Storage*

Variety	Method of Picking	Date Examined	Total No. Counted	Sound		Soft	
				Number	Percentage	Number	Percentage
Metallic Bell......	Water-raked	Nov. 27	536	439	81.9	97	18.1
Metallic Bell......	" "	" 27	555	438	78.9	117	21.1
Metallic Bell......	Dry-raked	" 27	318	284	89.3	34	10.7
Metallic Bell......	" "	" 27	282	266	94.3	16	5.7
Early Black......	Water-raked	" 27	540	465	86.1	75	13.9
Early Black......	" "	Dec. 2	605	527	87.1	78	12.9
Metallic Bell......	" "	" 3	703	598	85.1	105	14.9
Metallic Bell......	" "	" 3	574	491	85.5	83	14.5
Metallic Bell......	" "	" 3	608	525	86.3	83	13.7
Metallic Bell......	Dry-raked	" 3	582	522	89.7	60	10.3
Metallic Bell......	" "	" 3	651	590	90.6	61	9.4
Metallic Bell......	" "	" 3	510	485	95.1	25	4.5
Metallic Bell......	" "	" 3	590	540	91.5	50	8.5
Early Blackl......	Water-raked	" 3	696	595	85.5	101	14.5

Three other barrels of cranberries were also examined, one hand-picked and two water-raked. Of the latter, one was raked early while the berries were only slightly colored. The second was raked after the berries had become fully colored. The results are given in table 3.

TABLE 3. *A Comparison of the Keeping Quality of Hand-picked (dry) and of Water-raked Partly Colored (immature) and Fully Colored Metallic Bell Berries in Storage at Chicago, Ill., in 1918*

Method of Picking	Date Examined	Total No. Counted	Sound		Soft	
			Number	Percentage	Number	Percentage
Water-raked immature.....	Oct. 27	511	476	93.2	35	6.8
Water-raked mature.......	" 27	499	466	93.4	33	6.6
Hand-picked..............	" 27	439	421	95.9	18	4.1
Water-raked immature.....	Dec. 7	3,092	1,626	52.4	1,566	47.6
Water-raked mature........	" 7	3,174	2,147	68.2	1,027	31.8
Hand-picked..............	" 7	2,695	2,398	89.2	297	10.8

In this instance counts were made when the barrels first arrived at the Chicago warehouse and again about five weeks later. In the first examination only a single sample was taken from each barrel. On the later examination, however, samples were taken from the top, middle, and bottom of each barrel and the results were averaged. It is to be noticed that even on the first count the water-raked berries showed decidedly more soft berries than did the hand- (dry-) picked. The early-raked (partly colored) berries also showed a slightly higher percentage of soft berries than did those which were raked later after the berries were fully colored.

After a month in storage the differences were very apparent. The hand-picked berries had kept far better than the water-raked, and of the latter the dark berries had kept better than the light-colored ones. The better keeping quality of the mature, dark-colored berries may be accounted for by the fact that the respiratory activity of fruits decreases as the fruit ripens. Hence their oxygen requirement is less, and they suffer less injury through submergence than the less ripe berries, which have a higher oxygen requirement.

Counting tests were also made on several varieties of cranberries in storage at Minneapolis, Minn., during the winter of 1918. These berries were grown on the same bog and received essentially the same treatment after picking. The results are given in table 4.

TABLE 4. *A Comparison of the Keeping Quality of Dry- (hand-) picked and Water-raked Cranberries in Storage at Minneapolis, Minn., in 1918*

Variety	Method of Picking	Date Examined	Total Weight of Berries Examined	Sound		Soft	
				Weight	Percentage	Weight	Percentage
Searles Jumbo .	Water-raked[2]	Nov. 12	70 oz.	50 oz.	71.4[4]	20 oz.	28.6[4]
Searles Jumbo .	" "[2]	" 12	71 oz.	50 oz.	70.4[5]	21 oz.	29.6[5]
Searles Jumbo .	" "	" 12	67.5 oz.	55 oz.	81.5	12.5 oz.	18.5
Prolific.......	" "	" 15	58 oz.	43 oz.	74.2	15 oz.	25.8
Bennet Jumbo .	" "	" 15	66 oz.	48 oz.	72.7	18 oz.	27.3
Howes........	" "	" 5	66 oz.	60 oz.	90.9	6 oz.	9.1
Early Black....	" "	" 15	68 oz.	57 oz.	83.8	11 oz.	16.2
Searles Jumbo .	" "[2]	Dec. 9	1,689 g.	770 g.	45.6	919 g.	54.4
Searles Jumbo .	" "[3]	" 9	1,698 g.	1,063 g.	62.6	635 g.	37.4
Searles Jumbo .	" "[3]	" 9	1,858 g.	1,280 g.	68.9	578 g.	31.3
Searles Jumbo .	" "	" 9	1,921 g.	1,396.5 g.	72.7	524.5 g.	27.2
Searles Jumbo .	" "	" 9	1,979 g.	1,694 g.	85.6	285 g.	14.4
Searles Jumbo .	" "	" 10	1,894 g.	1,405 g.	74.2	489 g.	25.8
Searles Jumbo .	" "	" 10	1,832 g.	1,422 g.	77.6	410 g.	22.4
Searles Jumbo .	Dry-picked	" 10	2,151 g.	1,947 g.	90.5	204 g.	9.5
Searles Jumbo .	" "	" 10	1,944 g.	1,689 g.	86.9	255 g.	13.1
Searles Jumbo .	" "	" 10	2,050 g.	1,850 g.	90.2	200 g.	9.8
Searles Jumbo .	" "	" 11	2,030 g.	1,850 g.	91.1	180 g.	8.9
Searles Jumbo .	" "	" 11	1,691 g.	1,532 g.	90.6	159 g.	9.4
Searles Jumbo .	" "	" 11	1,931 g.	1,778 g.	92.1	153 g.	7.9
Howes........	Water-raked	" 11	1,893 g.	1,629 g.	86.1	264 g.	13.9
Howes........	" "	" 11	1,959 g.	1,694 g.	86.5	265 g.	13.5
Howes........	" "	" 11	1,846 g.	1,587 g.	86.0	259 g.	14.0
Howes........	" "	" 11	1,963 g.	1,733 g.	88.3	230 g.	11.7

[2] Picked early, berries not well colored, water held 2½ days, stored in barrels.

[3] Picked late, berries fully colored, water held 3 to 5 days, stored in barrels.

[4] From top of barrel.

[5] From center of barrel.

It may be seen by comparing the percentage of spoilage of water-raked berries from the same bog, stored under the same conditions, as shown in tables 1 and 4, that the extent of injury is not the same in all varieties. In table 1, comparing Metallic Bell and Bennet Jumbo, which were from the same grower and were examined at essentially the same time (November 23–25), it is seen that the Metallic Bell shows a greater percentage of spoilage than the Bennet Jumbo. A similar relation is indicated by the comparison of water-raked varieties examined at Minneapolis November 12–15 and also between Searles Jumbo and Howes examined December 9–11. In this case the test was a severe one, as the berries were under water from three to five days before being raked. Prolific and Bennet Jumbo were injured worse than Searles Jumbo, Howes least of all, and Early Black about the same as Searles Jumbo.

Causes of Spoilage in Water-raked Cranberries

The spoilage of water-raked cranberries is due to two causes: fungous rots and smothering. The fungi which are important in causing storage rots are present on the bogs and may gain entrance into the berries before they are picked. Having gained entrance, the fungus may remain inactive indefinitely, at least without any external manifestation of its presence, and then become active and result in the decay of the berry. It is possible also that infection may occur under certain conditions after the berries are picked. However this may be, it is well known that methods of handling and of storage are very important in determining the amount of spoilage.

Spoilage from smothering is not confined to water-raked berries. It may be caused by various conditions, as indicated by Shear and associates (4, p. 4). With the exception of smothering as a result of flooding, the conditions under which it occurs are the same for water-raked as for dry-raked berries. The length of time during which cranberries may be kept flooded without injury by smothering is determined by the oxygen content of the water and by the rate of respiration of the berries. The oxygen content of the water used for flooding Wisconsin marshes has been found to be generally low. For this reason injury by flooding is particularly apt to occur unless care is taken to prevent it. The causes of this oxygen deficiency will not be considered here. Some of the factors concerned have been indicated elsewhere (1) and will be treated more fully in another paper.

With reference to the rate of respiration, attention may be called to the age of the berries at the time of flooding in relation to the degree of injury sustained. Green cranberries are the first to suffer from smothering when flooded. This is well illustrated by the percentage of spoilage in partly colored and in fully colored water-raked berries as shown in table 3. A similar difference in the extent of injury between early-raked (not fully colored) and late-raked (fully colored) berries in storage at Minneapolis is shown in table 4. In order to avoid any effect of difference in treatment after picking, the comparison is limited to Searles Jumbo examined December 9. These berries were stored in barrels under the same conditions. The early-picked berries were in water not over 2½ days; the late-picked were in water 3–5 days. Notwithstanding this, the late-picked berries show a lower percentage of spoilage than the early-picked. Two barrels of the former showed 31.1 and 37.4 percent while the latter showed 54.4 percent of spoilage. The greater injury in the case of the early-picked berries is probably due to the higher respiration rate of these berries.

Methods of Drying in Relation to Spoilage

A large, if not the greater, part of the loss in cranberries after harvest is due to rot-producing fungi. The conditions favoring their development are not well understood. The prevalence of these fungi varies in different seasons, with different varieties, on different bogs, and even on different parts of the same bog. There seems to be a definite correlation with weather conditions, particularly with humidity. Because of the possibility of infection by fungi whenever sufficient moisture is present, water-raking may cause greater loss by decay of the berries after harvest than would be the case if they were dry-raked. Any difference in this respect between water-raked and dry-raked berries depends to some extent upon weather conditions. If there is danger of frost, the bog must be flooded whether the berries are to be water-raked or dry-raked. The only difference is that for the latter the water is withdrawn and the vines are allowed to become completely dry before the berries are raked. In this way complete drying is insured. In water-raking this may not be true, even with good drying conditions, unless care is taken. With poor drying conditions the berries may remain several days before becoming sufficiently dry to be taken into the storage house. In some instances the berries may be placed in drying crates and be taken at once into the storage house instead of being left outside to dry as is usual. In either case the period during which conditions are favorable for infection by fungi is greatly lengthened, and greater loss by fruit rots results.

Storage Conditions in Relation to Spoilage

This problem has been discussed by Shear and associates (4). Since all conditions which apply to dry-raked berries apply also to water-raked, it need not be treated at length here. The importance of keeping the berries dry during storage and in shipping is pointed out by these writers (p. 8). They also state that cranberries should be picked dry, as does Franklin (2, p. 216). None of the experiments described, however, show that picking wet is the cause of spoilage. The berries were not dried after picking but were placed in boxes and stored in a wet condition. Under these circumstances the increased spoilage must be attributed, largely at least, to increased infection by fungi as a result of slow or incomplete drying. This is confirmed by the following statement of Franklin (p. 203):

The averages of percentages in the table show that the berries stored wet rotted more than those stored dry in both series of tests. The wet berries in the second series were more nearly dry when picked than were those of the first series, this apparently accounting for the smaller difference in the average amounts of rot that developed in the two lots of Howes fruit. The wet berries left on the bog were perhaps dried a good deal, as compared with those housed at once, by the high temperatures and free circulation of the open air, this perhaps explaining their better keeping.

The necessity of ventilation during storage is indicated by a comparison of the keeping quality of water-raked berries stored in barrels and in boxes at Minneapolis. The following figures are taken from table 4. Two barrels of late-picked Searles Jumbo examined December 9 showed 31.1 and 37.4 percent of spoilage respectively. The average of the two barrels is 34.25 percent. On the same day and on the day following the examination of four lots of the same variety, late-picked, stored in boxes, showed an average spoilage of 22.5 percent. These lots were all of the same variety, were all late-picked, were held in water for the same length of time, and were stored under similar conditions except as to the kind of container in which they were packed. Therefore the difference in keeping quality must be attributed to the tightness of the barrels, which excluded oxygen sufficiently to cause smothering of the berries. A careful separation of smothered and fungus-rotted berries would probably have confirmed this view. Lack of time and facilities, however, prevented this from being done.

Whether or not there is a greater tendency of water-raked than of dry-raked berries to become smothered in barrels during storage cannot be stated at this time. It is possible, however, that the holding of water on the berries at picking time, especially if the water is held for a considerable period, interferes permanently with the normal respiration of the berries. This may have the effect of "weakening" the berries, or of producing a tendency to collapse earlier than would otherwise happen when subjected to somewhat unfavorable storage conditions. It has been demonstrated by Hill (3, p. 395) that peaches from which oxygen has been excluded for a few days were unable to regain their normal rate of respiration. He states that "this would indicate a permanent injury to the protoplasm or to some of the enzymes due to insufficient oxygen."

Precautions to be Observed in Water-raking

In view of the preceding discussion it may be said that water-raking of cranberries, without serious injury to their keeping quality, is possible under favorable conditions. Since most of the conditions are not controllable, the possibilities of injury by water-raking are greater than in dry-raking. There is no apparent reason, however, why injuries greater than those brought on by repeated or prolonged flooding for frost injury of bogs that are dry-raked, should be incurred if certain precautions are observed.

The first precaution in water-raking is to avoid holding water on the berries for a long period. This is particularly true of most Wisconsin bogs, as the oxygen content of the flooding water is low. A flooding period of 5 or 6 hours, or less if possible, is desirable. For this reason small flooding sections should be arranged. During the day, except in cloudy weather, injury is less apt to occur on account of the photosynthetic activity of the submerged vines. When there is danger of frost, the water may be held longer on account of the lower respiration rate of the cranberries, the reduction in the rate of oxidation of organic matter, and the increased capacity of the water for oxygen.

After picking, the conditions affecting the keeping quality of water-raked berries are the same as for dry-raked, and the recommendations of Shear and associates (4) should be carefully followed.

CONCLUSIONS

1. Storage tests indicate a superiority in the keeping quality of water-raked berries.
2. The spoilage of water-raked berries is due to two causes: fungous rots and smothering. Injury from smothering as a result of flooding is apt to occur in Wisconsin marshes on account of the low oxygen content of the flooding water.
3. Differences were observed in the keeping quality of different varieties of berries.
4. The age of the berries at the time of flooding is a factor in determining the degree of injury. Berries flooded before they are fully colored are more seriously injured than those flooded when fully colored.
5. The rate and completeness of drying affect the keeping quality by their influence on the extent of infection by rot-producing fungi. Quick and thorough drying is essential.
6. The possibilities of injury by water-raking are greater than in dry-raking. Water-raking may be done without serious injury if certain precautions are observed.
7. After picking, water-raked berries should be handled and stored with the same care given to dry-raked berries.

UNIVERSITY OF HAWAII,
HONOLULU

LITERATURE CITED

1. **Bergman, H. F.** The effect of cloudiness on the oxygen content of water and its significance in cranberry culture. Amer. Jour. Bot. **8**: 50–58. 1921.
2. **Franklin, H. J.** Report of the cranberry substation for 1916. Mass. Agr. Exp. Sta. Bull. 180. 1917.
3. **Hill, G. R., Jr.** Respiration of fruits and growing plant tissues in certain gases, with reference to ventilation and fruit storage. Cornell Agr. Exp. Sta. Bull. 330. 1913.
4. **Shear, C. L., Stevens, N. E., Wilcox, R. B., and Rudolph, B. A.** Spoilage of cranberries after harvest. U. S. Dept. Agr. Bull. 714. 1911.

INCIPIENT DRYING AND WILTING AS INDICATED BY MOVEMENTS OF COCONUT PINNAE[1]

Sam F. Trelease

(Received for publication September 12, 1921)

The amount of water in the plant at any time depends upon the relation between the rates of absorption and of transpiration that have recently been in operation. During the day most plants lose water more rapidly than they absorb it; in the night, on the other hand, plants usually absorb water more rapidly than they transpire it. Thus it is generally true that the water content of the plant is high in the night, especially after midnight, while it is lower during the day, especially in the afternoon; an incipient drying, or saturation deficit, of plant tissues generally appears early in the day and becomes progressively greater as the day advances. Incipient drying is accompanied by decreasing turgor in some or all of the leaf tissues and frequently results in actual wilting (Livingston and Brown, 7; Livingston, 6). This diurnal deficit appears to be one of the most important features in the complex relation of the plant to its water supply; and it has an important bearing on the general problems of drought resistance and irrigation of cultivated crops.

A method employed by Livingston and Brown (7) in studying the changes that occur in the relative water content of leaves throughout the day and night, is to gather a large number of similar leaves from hour to hour and to determine the moisture content of each lot as percentage of the dry or green weight. As pointed out by these workers, this method fails to take into account the small diurnal increase in materials other than water within the tissues; if the water content *per leaf* remained constant, such accumulation would of course *lower* the *percentage* of water on the basis of weight; but they regard the percentage changes shown by their data as mainly due to lowered water content per leaf, brought about when the ratio of the rate of water loss to that of water supply became less than unity. Similar results for the twigs of a desert plant were obtained by Edith B. Shreve (14).

Other workers (Lloyd, 10; Miller, 11) have employed circular leaf samples of known area, instead of entire leaves, and have calculated the water percentage on the basis of leaf area. The use of such samples makes it possible to study changes in weight that may be due to the accumulation of material other than water, but it does not permit a study of alterations due to changes in leaf area; according to Thoday (16), during wilting some kinds of leaves may shrink two or three or in some cases as much as six percent. A diminished water content in the plant as a whole has been studied also, by determining the difference between the rate of transpiration and that of absorption, as these rates vary independently throughout the day and night (Renner, 12, 13).

---

[1] Botanical publication from the Johns Hopkins University, no. 71.

The present paper describes preliminary experiments that deal with what may offer another method, useful with some plants, for the indirect study of incipient drying; this method depends upon changes in leaf position or leaf shape, resulting from changes in leaf water content and concomitant alterations in the turgidity relations of different leaf tissues.

Leaves of the coconut (*Cocos nucifera*) were employed. The leaf of this plant is primarily to be regarded as a very large, entire or merely notched leaf with very regular pinnately arranged veins reaching laterally outward from a central rib. As it develops, the leaf blade tears, however, midway between each pair of adjacent veins, so that it comes to have the appearance of a pinnately compound leaf, the true midrib appearing like a rachis and the lateral veins appearing like the midribs of pinnae. The lateral strips, separated by the tearing just mentioned, will be called pinnae in this paper.

It was noted by Copeland (2) that running ventrally for its entire length along each side of the midrib of the coconut pinna there is a narrow, colorless strip, the two strips together constituting a "hinge." Through the action of the hinge the two wings of the pinna may take various positions, thus altering the general configuration of the pinna. As Copeland has pointed out, when the leaves are well supplied with water the hinge cells are distended and the two pinna wings are held nearly in the same plane, like the right and left halves of an ordinary leaf; but when there is a deficiency of water in the hinge tissue the two pinna wings revolve downward, about the pinna midrib as an axis, so that their lower faces approach each other. When the pinnae are on the point of beginning to curl on account of drying, the angle between the two wing faces is about 25 degrees of arc. The total actual width of an average coconut pinna is about 3.5 cm., the wing width being half as great. Since the two wings remain approximately flat until curling begins, simply moving upward or downward on the midrib as an axis, the angle between them may be conveniently approximated by means of the distance between the two free, parallel edges. The behavior of the pinna hinges is illustrated by Copeland's (2) tables showing variations, for several days, in the distance between the edges of pinnae.

Since the action of the hinges and the resulting "opening" or "closing" of the pinnae appeared to be related to turgidity changes, it seemed desirable to study the movements with reference to the water content of the pinnae and to the temperature and evaporating power of the air. The present paper reports a preliminary study of this kind.

These experiments were carried out at the College of Agriculture of the

University of the Philippines, at Los Baños, during April and May, 1918, in connection with class work in plant physiology. It is a pleasure to acknowledge indebtedness to Prof. B. E. Livingston for suggestions in the preparation of this paper; and to members of the class, especially Mr. Pedro David and Mr. F. de Peralta, for assistance in securing the measurements.

Experimentation

Excised pinna samples. To study the relation between water content and the degree of closure, pinnae were cut from a plant and taken to the laboratory, where the measurements were made. From near the middle of a pinna three 8-cm. lengths were cut. The pieces were weighed at intervals, the distance between the two free edges of each piece (here called its apparent width) being measured immediately after each weighing. After continuing the measurements for several hours, the area of one side of each piece was determined. They were then dried, and the water content was calculated for each weighing.

The results of five similar tests, made in the laboratory (temperature, 27° C.) with excised pieces of pinnae, are presented in table 1, three pieces being used in each test. From the average apparent width of the pinna in each case has been derived the average magnitude of the angle between the two lower faces of the pinna wings; and the average angular magnitudes are given, for the several observations, in the last column of the table; this value may be called the angle of pinna divergence, which is taken as related to the turgor condition of the two pinna hinges.

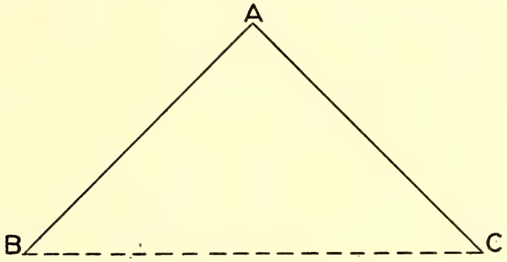


Fig. 1. Diagrammatic cross section of coconut pinna; lines *AB* and *AC* representing the two pinna wings, hinged to the midrib at *A*, and the line *BC* representing the apparent pinna width.

If the diagram of figure 1 represents a cross section of a coconut pinna, lines *AB* and *AC* representing the two pinna wings, hinged to the pinna midrib at *A*, then line *BC* represents the apparent pinna width for the cross section used. Determination of the average apparent width of the pinna sample used was made at each time of observation, and the apparent-width values are given in column 6 of the table. Determination of the average width of each of the two (similar) wings was also made for each pinna sample, the value being taken to be constant for all observations. Returning to the diagram of figure 1, the quotient obtained by dividing half the length of line *BC* by the length of line *AB* (or by that of line *AC*, since these are equal) is the sine of half the angle *BAC*. From the sine is readily determined the magnitude of the half-angle itself (by use of a table of sine values), and multiplying this value by 2 gives the magnitude of the whole angle *BAC*, the angle of divergence of the two pinna wings. The last named magnitude, being the average for the pinna sample used, is the one given in the last column of the table, for each observation.

TABLE 1. *Relation between Water Content of Excised Portions of Pinnae and Average Angle of Divergence between the Two Pinna Wings*

Test No. and Values that Are Constant for the Test	Time Elapsed since Excision	Ave. Green Weight per 100 Sq. Cm. of Area (One Side)	Ave. Water Content per 100 Sq. Cm. of Area (One Side)		Ave. Apparent Width of Pinna Sample	Ave. Magnitude of Angular Divergence between Pinna Wings
			Actual (from Weight and Area of Samples)	Calculated from Equation of Smoothed Rectilinear Graph (see Discussion)		
Test I	*min.*	*grams*	*grams*	*grams*	*cm.*	*deg. of arc*
Ave. actual width of pinna wing, 1.73 cm. Ave. area of pinna sample (one side), 27.7 sq. cm. Ave. dry weight per 100 sq. cm. of area (one side), 1.8472 g. Equation constants (see discussion), $K = .3191$; $L = 2.2272$.	0	4.7410	2.8938	2.8877	2.070	73° 30′
	5	4.7283	2.8811	2.8769	2.036	72° 6′
	10	4.7165	2.8693	2.8686	2.010	71° 2′
	15	4.7057	2.8585	2.8632	1.993	70° 20′
	20	4.6989	2.8517	2.8549	1.967	69° 18′
	25	4.6883	2.8411	2.8472	1.943	68° 20′
	30	4.6818	2.8346	2.8335	1.900	66° 36′
	35	4.6753	2.8281	2.8303	1.890	66° 12′
	65	4.6267	2.7795	2.7824	1.740	60° 24′
	95	4.5733	2.7261	2.7208	1.547	53° 8′
	125	4.5187	2.6715	2.6685	1.383	47° 8′
	155	4.4619	2.6147	2.6155	1.217	41° 12′
	185	4.3982	2.5510	2.5485	1.007	33° 50′
	215	4.3414	2.4942	2.4994	0.853	28° 32′
Test II Ave. actual width of pinna wing, 1.73 cm. Ave. area of pinna sample (one side), 27.9 sq. cm. Ave. dry weight per 100 sq. cm. of area (one side), 1.8140 g. Equation constants (see discussion), $K = .2825$; $L = 1.6262$.	0	4.1899	2.3759	2.3825	2.677	101° 22′
	30	4.1239	2.3099	2.3070	2.410	88° 18′
	60	4.0711	2.2571	2.2570	2.233	80° 22′
	90	4.0171	2.2031	2.2017	2.037	72° 8′
	120	3.9571	2.1431	2.1488	1.850	64° 40′
	150	3.8957	2.0817	2.0839	1.620	55° 50′
Test III Ave. actual width of pinna wing, 1.71 cm. Ave. area of pinna sample (one side), 27.5 sq. cm. Ave. dry weight per 100 sq. cm. of area (one side), 1.7225 g. Equation constants (see discussion), $K = .2628$; $L = 1.6338$.	0	4.0369	2.3144	2.3145	2.590	98° 52′
	30	3.9813	2.2588	2.2558	2.367	87° 56′
	60	3.9330	2.2105	2.2164	2.217	81° 6′
	90	3.8867	2.1642	2.1620	2.010	72° 14′
	120	3.8377	2.1152	2.1139	1.827	64° 48′
	150	3.7875	2.0650	2.0656	1.643	57° 38′

Table 1—*Continued*

Test No. and Values that Are Constant for the Test	Time Elapsed since Excision	Ave. Green Weight per 100 Sq. Cm. of Area (One Side)	Ave. Water Content per 100 Sq. Cm. of Area (One Side)		Ave. Apparent Width of Pinna Sample	Ave. Magnitude of Angular Divergence between Pinna Wings
			Actual (from Weight and Area of Samples)	Calculated from Equation of Smoothed Rectilinear Graph (see Discussion)		
	min.	*grams*	*grams*	*grams*	*cm.*	*deg. of arc*
Test IV Ave. actual width of pinna wing, 1.56 cm. Ave. area of pinna sample (one side), 24.9 sq. cm. Ave. dry weight per 100 sq. cm. of area (one side), 1.7861 g. Equation constants (see discussion), $K = .3457$; $L = 2.1740$.	0	4.5473	2.7612	2.7558	1.683	65° 18′
	30	4.4870	2.7009	2.7074	1.543	59° 16′
	60	4.4333	2.6472	2.6580	1.400	53° 20′
	90	4.3837	2.5976	2.6106	1.263	47° 46′
	120	4.3363	2.5502	2.5681	1.140	42° 52′
	150	4.2834	2.4973	2.4920	0.920	34° 18′
	180	4.2357	2.4496	2.4392	0.767	28° 28′
Test V Ave. actual width of pinna wing, 1.66 cm. Ave. area of pinna sample (one side), 26.6 sq. cm. Ave. dry weight per 100 sq. cm. of area (one side), 1.5022 g. Equation constants (see discussion), $K = .1718$; $L = 1.6021$.	0	3.4292	1.9270	1.9268	1.890	69° 24′
	5	3.4186	1.9164	1.9153	1.823	66° 36′
	10	3.4028	1.9006	1.9084	1.783	64° 58′
	13	3.4023	1.9001	1.9038	1.756	63° 52′
	18	3.3915	1.8893	1.8912	1.683	60° 56′
	23	3.3785	1.8763	1.8787	1.610	58° 0′
	28	3.3897	1.8675	1.8706	1.563	56° 10′
	30	3.3634	1.8612	1.8677	1.546	55° 30′
	36	3.3559	1.8537	1.8505	1.446	51° 38′
	43	3.3428	1.8406	1.8419	1.396	49° 44′
	47	3.3355	1.8333	1.8375	1.370	48° 44′
	50	3.3303	1.8281	1.8282	1.316	46° 42′
	58	3.3207	1.8185	1.8156	1.243	43° 58′
	64	3.3094	1.8072	1.8059	1.186	41° 52′

The equation constants given in column 1 and the calculated values of column 5 will be considered below.

Pinna still attached. For the study of angular changes in the pinnae still attached to the plant, ten pinnae were selected on each of three trees (thirty pinnae in all), and these were measured at hourly intervals, from 6 a.m. to 6 p.m., for six days. Ink marks on the pinna edges insured that the measurements would always be made at the same place. A shaded thermometer near the plants was read at the times of measurement, and a Livingston white spherical atmometer, unshaded and also near the plants, was likewise read.

The data thus secured are shown in table 2.

Discussion of Results

Excised pinna samples. The results presented in table 1 show that excised pinnae in laboratory tests gradually lost weight as the result of transpiration unaccompanied by absorption, and that at the same time the apparent pinna width gradually decreased. To study the relation between apparent pinna width and foliar water content, the apparent-width values (table 1, column 6) were plotted as abscissas of a graph and the average actual water contents (table 1, column 4) were plotted as ordinates. The five graphs obtained in this manner were all approximately straight lines, suggesting a linear relationship between the water content per unit of

TABLE 2. *Hourly Fluctuations in Apparent Pinna Width (for Pinnae Attached to the Plant), together with Hourly Temperature and Evaporation Data*

Time of Observation	Ave. Apparent Pinna Width (10 Pinnae on Each Plant)			Temperature	Evaporating Power of the Air (White Spherical Atmometer)
	Plant *A*	Plant *B*	Plant *C*		
	cm.	*cm.*	*cm.*	*deg. C.*	*cc. per hr. for preceding hour*
April 29					
6 a.m.	1.94	1.91	2.32	22	
7 a.m.	1.81	1.82	2.05	27	0.8
8 a.m.	1.76	1.73	1.97	29	1.6
9 a.m.	1.70	1.69	1.96	30	1.9
10 a.m.	1.59	1.68	1.87	31	1.7
11 a.m.	1.63	1.65	1.87	31	3.3
12 noon	1.56	1.68	1.80	33	3.6
1 p.m.	1.54	1.68	1.79	35	4.9
2 p.m.	1.50	1.50	1.97	37	4.4
3 p.m.	1.46	1.58	1.88	37	5.1
4 p.m.	1.65	1.57	1.86	34	3.6
5 p.m.	1.78	1.65	1.87	30	3.8
6 p.m.	1.87	1.75	1.90	28	1.4
April 30					
6 a.m.	1.96	1.96	2.26	23	
7 a.m.	1.84	1.83	2.00	28	0.9
8 a.m.	1.76	1.73	1.97	30	1.2
9 a.m.	1.77	1.66	1.94	32	2.0
10 a.m.	1.69	1.58	1.90	33	3.2
11 a.m.	1.52	1.45	1.81	31	2.7
12 noon	1.41	1.46	1.79	33	2.9
1 p.m.	1.50	1.48	1.77	35	4.1
2 p.m.	1.50	1.59	1.86	35	4.9
3 p.m.	1.70	1.55	1.89	30	5.0
4 p.m.	1.55	1.48	1.86	33	3.4
5 p.m.	1.68	1.70	1.86	30	4.2
6 p.m.	1.80	1.75	1.91	27	3.1
May 2					
6 a.m.	2.00	1.90	2.16	25	
7 a.m.	1.92	1.79	2.06	26	0.7
8 a.m.	2.01	1.91	2.29	29	(a)
9 a.m.	1.89	1.86	2.08	27	(a)
10 a.m.	1.89	1.78	2.05	29	(a)
11 a.m.	1.73	1.66	1.95	30	0.8
12 noon	1.66	1.58	1.94	33	2.0
1 p.m.	1.62	1.46	1.93	34	3.3
2 p.m.	1.59	1.49	1.99	34	4.1
3 p.m.	1.68	1.44	1.99	35	4.4
4 p.m.	1.62	1.51	1.99	35	4.0
5 p.m.	1.58	1.43	2.01	32	4.0
6 p.m.	1.66	1.57	2.03	27	3.1

[a] Rainfall during these hours.

TABLE 2—*Continued*

Time of Observation	Ave. Apparent Pinna Width (10 Pinnae on Each Plant)			Temperature	Evaporating Power of the Air (White Spherical Atmometer)
	Plant *A*	Plant *B*	Plant *C*		
	cm.	*cm.*	*cm.*	*deg. C.*	*cc. per hr. for preceding hour*
May 3					
6 a.m......	2.07	2.03	2.18	23	
7 a.m......	1.93	1.86	2.12	27	0.7
8 a.m......	1.78	1.67	2.06	28	1.2
9 a.m......	1.75	1.57	1.98	30	1.1
10 a.m......	1.59	1.49	1.92	32	1.9
11 a.m......	1.58	1.51	2.00	32	3.0
12 noon.....	1.57	1.42	1.89	35	3.4
1 p.m......	1.55	1.42	1.94	36	4.6
2 p.m......	1.59	1.43	2.00	33	3.8
3 p.m......	1.69	1.45	2.06	31	4.0
4 p.m......	1.63	1.40	1.90	31	2.3
5 p.m......	1.74	1.48	1.93	31	2.1
6 p.m......	1.82	1.59	2.03	30	1.5
May 4					
6 a.m......	2.02	1.90	2.06	24	
7 a.m......	1.74	1.75	2.02	25	0.9
8 a.m......	1.73	1.66	1.98	30	0.4
9 a.m......	1.66	1.50	1.99	29	2.1
10 a.m......	1.69	1.48	1.97	33	2.0
11 a.m......	1.66	1.47	1.97	34	3.5
12 noon.....	1.55	1.47	1.97	35	4.1
1 p.m......	1.52	1.35	1.91	36	6.9
2 p.m......	1.48	1.58	1.87	34	3.3
3 p.m......	1.62	1.62	1.76	33	3.3
4 p.m......	1.60	1.46	1.85	31	4.4
5 p.m......	1.68	1.50	1.89	31	4.1
6 p.m......	1.73	1.58	1.96	30	3.5
May 6					
6 a.m......	2.02	1.98	2.17	24	
7 a.m......	1.90	1.77	1.96	28	0.7
8 a.m......	1.70	1.60	1.96	28	1.4
9 a.m......	1.71	1.51	1.88	30	2.0
10 a.m......	1.67	1.50	1.81	32	([b])
11 a.m......	1.48	1.51	1.79	34	([b])
12 noon.....	1.52	1.39	1.79	32	3.8
1 p.m......	1.52	1.31	1.88	34	3.9
2 p.m......	1.50	1.33	1.92	35	4.0
3 p.m......	1.52	1.30	1.83	37	5.0
4 p.m......	1.48	1.33	1.94	35	4.6
5 p.m.	1.43	1.28	1.94	33	4.8
6 p.m......	1.55	1.35	2.03	32	1.7

[b] Accident prevented atmometer record.

area and the apparent pinna width. A smoothed graph, being a straight line, was then drawn for each of the five tests. The equations for the five rectilinear graphs have the general form, $y = Kx + L$, in which y represents the water content in grams per 100 sq. cm. of area (one side), x represents the apparent pinna width in centimeters, K is the slope constant of the graph, and L is another constant representing the value that y would have if the two pinna wings were in contact (the angle between them being then zero), assuming that the rectilinear relation shown by the graph in question were to hold at this stage of wilting or closing of the hinges. The values of K and L for each of the five equations are given in column 1 of table 1, and are also brought together below:

Test No.	K	L
1	.3191	2.2272
2	.2825	1.6262
3	.2628	1.6338
4	.3457	2.1740
5	.1718	1.6021

After these equation constants had been derived for each test, the water-content value was calculated, by means of the constants for the test in question, for each apparent-width value given in table 1, and the "calculated" water-content values are shown in column 5 of the table. The close agreement between the corresponding values of columns 4 and 5 shows how nearly rectilinear the relation between water content and apparent pinna width actually was for these tests. The disagreements are generally insignificant, and it seems safe to conclude that the linear equation form represents a true relation for these pinna samples. While there are considerable differences between the values for K and between those for L in the five tests (these differences being perhaps due to differences in physiological state between the several lots of pinna samples, which were selected at random), it may be stated that, in a general way, the values of the two constants of proportionality for this relation of coconut pinnae may be considered as: K, 0.3; L, 2.0. If this statement approximates the general truth, then the water content (in grams) for 100 sq. cm. of leaf surface (one side) is numerically about equal to 2 *plus* one third of the apparent pinna width (in centimeters); $y = 2 + 0.3x$.

Referring again to the diagram of figure 1, the apparent pinna width—which seems to be directly related to the foliar water content, as just noted—is itself truly proportional to the sine of half of the angle of divergence between the two pinna wings, the two wings being of like width, practically constant after the pinna has ceased to increase in size. This half-angle deserves special attention, for it represents the degree of divergence of either pinna wing from the position that this wing would have if it were in contact with the other wing—when the pinna would be completely "closed." As has been mentioned, each wing is provided with its own hinge, where it joins the pinna midrib. The half-angle here considered is clearly a measure of the turgidity conditions in the hinge cells, although the half-angle does not entirely vanish when all turgidity disappears, the wings not coming into complete contact even when the pinna is strongly wilted.

Since the sine of the half-angle just considered is directly related to the apparent pinna width, and since the foliar water content is similarly related to this width, it follows that the water content is likewise related to the sine in question. Considering the half-angle as A (the angular divergence of one pinna wing from its vertical or central position, this being one half of the angle of divergence between the *two* wings), the relation between foliar water content and the magnitude of angle A is shown by the following general equation of proportionality:

$$y = M \text{ sine } A + L,$$

in which y and L have the meanings given above and M is a new slope constant. The values of M for the five tests with excised pinna samples are shown below:

Test 1	Test 2	Test 3	Test 4	Test 5
1.1041	0.9775	0.8961	1.0786	0.5704

Generalizing in a very rough way, it may be said that M is about unity, and that the water content of these leaves (in grams per 100 sq. cm. of area, one side) is numerically about equal to the sine of the angle of single-wing divergence *plus* 2.

Pinnae still attached. Opportunity was not presented for the testing of the quantitative relations between foliar water content and position of the pinna wings with pinnae still attached to trees in the open. As table 2 shows, the records of hourly changes in apparent pinna width throughout the day are useful in forming a picture of the diurnal march of these changes and, presumably, of the turgor fluctuations in the hinge cells, upon which the position of the pinna wings depends. The maximum distance between the edges of the pinna occurred, on each day except May 2, at 6 a.m., the time of the earliest test, thus indicating that the hinge cells were more completely saturated with water at this hour than at any other hour at which tests were made. The occurrence of the maximum at 8 a.m. on May 2 is probably related to a fall of rain that occurred between 7 and 8 o'clock on that day. By 7 a.m. on most days the pinna wings had already begun to droop, and the angle of divergence continued to decrease, hour by hour, reaching a minimum value at some time between 11 a.m. and 5 p.m., the minimum width usually occurring between 1 p.m. and 3. If Copeland's (2) interpretation of this phenomenon and the indications suggested by the present tests with excised pinnae represent what happens, it may be supposed that the greatest incipient drying occurred usually between 1 p.m. and 3. After reaching the minimum the pinnae began slowly to open, and by 6 p.m. they had usually expanded to about the condition shown for 7 a.m. or 8. Since other tests, continued throughout the night, showed that the maximum apparent width was reached some time early in the morning, and that it had begun to decrease somewhat by 6 a.m., when the tests here reported were begun, it is suggested that the maximum water content of the hinge cells probably usually occurs during the hours of darkness, very early in the morning. The observed fact that the growth rate of coconut is usually higher in the night than during the day is apparently related to higher water content of the plant as a whole in the night—at least to greater turgidity in the enlarging parts.

A comparison of the hours of occurrence of the minimum expansion for the three sets of pinnae measured (three different plants) shows that there was no constant relationship between the three sets as to the time of greatest drooping of the wings, though the minimum for plant *C* usually occurred earlier in the day than did the minima for the other two plants. From this variation it again appears (as was suggested by the tests with excised pieces) that individual differences between plants or leaves may result in somewhat different responses in incipient drying and wilting. The temperature and evaporation data given in table 2 show that in these tests the maximum temperature occurred between 1 p.m. and 4, usually between 1 p.m. and 3; and that the maximum evaporation rates occurred between noon and 3 p.m., most frequently about 2 p.m. or 3. Minimum temperature is shown in all cases for 6 a.m., and the minimum evaporation rate is shown for each day (except during rainfall on May 2) as occurring between 7 a.m. and 8. Had night readings been taken, they would undoubtedly have shown lower rates of evaporation than any during the day. A comparison of the recorded fluctuations in temperature and evaporation with corresponding fluctuations in wing position shows that the minimum leaf width usually occurred within one or two hours of the occurrence of the maximum temperature and the maximum evaporating power of the air, but that there was no very constant relation between these variations. If pinna expansion depends upon the turgidity and water content of the hinge cells, it would not be expected that a simple relationship would exist between either temperature or evaporation and angular divergence, since these external conditions affect the water content of only indirectly effective cells, by influencing the rate of water loss from the leaves and that of water absorption by the roots.

The hourly variations in the wing position for these coconut leaves resemble similar variations that other workers have observed in water content and wilting for many other kinds of plants. Thus the minimum water content observed by Livingston and Brown (7) occurred usually within an hour or two of the time of highest evaporation rates, the minimum moisture content of the leaves of most of their plants occurring between 1 p.m. and 5. Lloyd (9) found that the water content of the leaves of *Fouquieria splendens* began to decrease at daybreak and reached a minimum some time between noon and 4 p.m.; after that time the water content increased until about 4 a.m. With the cotton plant Lloyd (10) found that the minimum water content of leaves was reached at about 2 p.m. Miller (11), studying maize, milo, and kafir, found that all three of the plants showed a decreased water content between 7 a.m. and 11; in one third of his observations on maize and milo and in one fifth of those on kafir, the leaves showed a gain in their leaf water content between 11 a.m. and 1 p.m. And from 1 p.m. to 3 the leaves gained in water content in one half of his observations on maize and milo and in three fourths of those on kafir, while from 3 p.m. to 5 the leaves of kafir showed a gain in water in all observations.

In the case of *Cestrum nocturnum*, Brown and Trelease (1) found that young shoots wilted and actually decreased in length, instead of elongating, on dry days during the time they were exposed to direct sunlight; in the night such shoots elongated rapidly, but during the day they showed no elongation excepting after they had returned to their original length late in the afternoon. Absence of growth and actual shrinking were apparently connected with excessive transpiration, which caused the plants to lose water more rapidly than they absorbed it. The movements of coconut pinnae appear to be similar also to the shrinking of tree trunks observed by Kraus (5), of fruits observed by Darwin (4) and by Smith (15), and of leaves observed by Thoday (16). Thoday found that leaves may shrink in area as much as six percent. During periods of intense sunshine, the minimum area usually occurred at about noon, and alternating periods of cloudiness and sunshine during the middle of the day were accompanied by prompt increases and decreases, respectively, in leaf area. The reversible movements of coconut pinnae are apparently due to alterations in the moisture content of the thin-walled "hinge" cells that lie in a row at either side of the midrib, the "hinge" cells apparently changing readily in shape or size, or both, with even slight variations in their water content. But it is not to be expected that fluctuations in the average water content of the whole pinna would be accurately and promptly reflected in turgidity changes and in resulting movements of the hinge tissues. There may be a considerable *lag* between leaf movements and alterations in the general foliar moisture, and the hinge cells may be peculiarly sensitive to alteration in the relation between transpiration and water supply. The possible action of light or temperature as a stimulus, high rates of evaporation, or changes in carbohydrate or acid content of the hinge cells or of the foliar tissues, etc., may also affect the relationship between the hinge tissue and the rest of the pinna. The occurrence of saturation deficit in plant tissues follows periods during which transpiration rates have exceeded rates of absorption. The physiological importance of such deficits has been emphasized by Livingston and Hawkins (8), who point out the possibility that the critical value of the ratio of transpiration to absorption (indicating the tendency of the plant to have its moisture content reduced) at which growth or other vital activity may be definitely affected, may eventually become recognized as a physiological and ecological constant, by which some of the over-discussed "adaptations" of plants may be quantitatively stated, at least in an approximate way.

Livingston and Brown (7) have suggested that in the diurnal minimum in the water content of foliage leaves we may have a criterion of some importance to scientific agriculture, at least for arid regions, since by this criterion it may be possible to determine, indirectly, the status of the water relations of the plant, and to foresee the need of increased soil moisture, long before the usual criterion of cessation of growth or actual wilting becomes manifest. The movements of coconut leaves considered in this paper suggest a possible use of such changes in leaf position in connection with practical agriculture. It seems very important to have easily determined quantitative methods by which the condition of a crop may be judged, as a basis for proper methods of cultivation. The most convenient general index of the health of the plant, according to Copeland (3), is the growth rate. Although such indices are very much needed, agronomy and plant physiology have furnished few that may be conveniently applied, mere general inspection usually having to serve as the basis for judging the condition of a crop. If changes in the pinna movement of coconut, or similar changes in the leaves of abaca and banana (which have been observed by the writer), represent changes in water content of the leaf tissues, measurements of these may prove to be of value in connection with the practice of irrigation; the degree of movement or its duration, the hour of greatest closing or of maximum expansion, or the range between maximum and minimum apparent width, may be of more value as a criterion for irrigation practice than mere observations on the appearance of the plant or of the soil. The yields of such plants as coconut, abaca, and banana might thus be increased considerably by a judicious use of irrigation, even in localities having apparently suitable, but not optimal, moisture conditions. It may even be possible to use plants exhibiting such leaf movements as indicators for the irrigation or cultivation of other kinds of crop plants whose leaves do not show reversible movements correlated with their moisture content.

BIBLIOGRAPHY

1. **Brown, W. H., and Trelease, S. F.** Alternate shrinkage and elongation of growing stems of *Cestrum nocturnum*. Philip. Jour. Sci. C, **13**: 353–360. 1918.
2. **Copeland, E. B.** On the water relation of the coconut palm (*Cocos nucifera*). Philip. Jour. Sci. **1**: 6–57. 1906.
3. ——. Experiments on the coconut I. Philip. Agric. and For. **3**: 121–126. 1914.
4. **Darwin, F.** On the growth of the fruit of Cucurbita. Annals of Bot. **7**: 459. 1893.
5. **Kraus, G.** Physiologisches aus den Tropen. Ann. Jard. Bot. Buitenzorg **11**: 196. 1895.
6. **Livingston, B. E.** Incipient drying and temporary and permanent wilting of plants, as related to external and internal conditions. Johns Hopkins Univ. Circ. **293**: 176–182. 1917.
7. ——, **and Brown, W. H.** Relation of the daily march of transpiration to variations in the water content of foliage leaves. Bot. Gaz. **53**: 309–330. 1912.
8. ——, **and Hawkins, L. A.** The water-relation between plant and soil. Carnegie Inst. Wash. Publ. **204**: 5–48. Washington, D. C., 1915.
9. **Lloyd, F. E.** The relation of transpiration and stomatal movements to the water content of the leaves of *Fouquieria splendens*. Plant World **15**: 1–14. 1912.
10. ——. Leaf water and stomatal movement in Gossypium, and a method of direct visual observation of stomata *in situ*. Bull. Torr. Bot. Club **40**: 1–26. 1913.

11. **Miller, E. C.** Daily variation of water and dry matter in the leaves of corn and the sorghums. Jour. Agr. Res. **10**: 11–45. 1917.

12. **Renner, O.** Experimentelle Beiträge zur Kenntnis der Wasserbewegung. Flora **103**: 171–247. 1911.

13. ——. Versuche zur Mechanik der Wasserversorgung I. Der Druck in den Leitungsbahnen von Freilandpflanzen. Ber. Deutsch. Bot. Ges. **30**: 576–580. 1912.

14. **Shreve, Edith B.** The daily march of transpiration in a desert perennial. Carnegie Inst. Wash. Publ. 194. Washington, D. C., 1914.

15. **Smith, A. M.** On the application of the theory of limiting factors to measurements and observations of growth in Ceylon. Ann. Roy. Bot. Gard. Peradeniya **3**: 303. 1906.

16. **Thoday, D.** Experimental researches on vegetable assimilation and respiration V. A critical examination of Sachs' method for using increase of dry weight as a measure of carbon dioxide assimilation in leaves. Proc. Roy. Soc. London B, **82**: 1–55. 1909.

DR. W. J. BEAL'S SEED-VIABILITY EXPERIMENT

H. T. Darlington

(Received for publication September 22, 1921)

In the autumn of 1879, Dr. Beal began an experiment to test the viability of the seeds of some of the more common plants growing in the vicinity of the Agricultural College at East Lansing, Michigan. Most of the seeds were those of common weeds. The first account of the experiment appeared in the *Botanical Gazette* for August, 1905. This gave the results for the first twenty-five years. In the 17th Report of the Michigan Academy of Science (1915) the results were published up to the fall of 1914.

The nature of the experiment is best indicated by Dr. Beal himself, from whom I quote. He says:

> I selected fifty freshly grown seeds from each of twenty-three different kinds of plants. Twenty such lots were prepared with the view of testing them at different times in the future. Each lot or set of seeds was well mixed in moderately moist sand, just as it was taken three feet below the surface, where the land had never been plowed. The seeds of each set were well mixed with the sand and placed in a pint bottle, the bottle being filled and left uncorked, and placed with the mouth slanting downwards so that the water could not accumulate about the seeds. These bottles were buried on a sandy knoll in a row running east and west and placed fifteen paces northwest from the west end of the big stone set by the class of 1873. A boulder stone, barely even with the surface soil, was set at each end of the row of bottles, which were buried about 20 inches below the surface of the ground.

As table 2 shows, a bottle containing a set of seeds has been dug up every five years and tested for germination. Results are now at hand for forty years, and there are still twelve sets buried, or enough to last sixty more years if the same rate of testing is followed. Dr. E. A. Bessey, under whose direction the last two tests have been made, has suggested that the interval of testing be lengthened to ten years, thus increasing by sixty years the duration of the experiment.

Dr. Beal made all the tests up to and including 1909, the thirtieth year. The tests fall due in the fall, and the 1914 test was made at that time. The last test, however, was made in the spring of 1920, because the test had been delayed until the ground had frozen so hard as to make it impossible to dig up a bottle. The rather unusual results of the last test, however, have suggested that the wintering-over due to this delay has been rather fortunate than otherwise. The showing made was much better than that of 1914. Several factors may have contributed to this.

The method of testing the last time, which was essentially that of 1914, was as follows: The equipment consisted of two flats about 18 x 20 x 2½ inches deep. These were nearly filled with rich sifted loam, sterilized at

10–12 pounds' pressure for three hours. One of the flats was used for growing check seeds of the same species which had succeeded in germinating during the last fifteen or twenty years. With checks of this kind it is very easy to identify very young seedlings, which is indispensable for recording the number that start to germinate. The flats being in readiness, the sand from the bottle dug up was spread evenly over the surface in one of the flats and mixed slightly. Seeds were at once planted in the other flat and labeled, each species being kept in a small area of its own. This was on March 27. The flats were kept in a semi-shady place in the greenhouse at a temperature of about 22° C. They were watered once daily with a fine spray. The species planted in the check flat were as follows, being arranged here in the order in which they came up: *Brassica nigra*, *Bursa bursa-pastoris*, *Anthemis cotula*, *Alsine media*, *Lepidium virginicum*, *Amaranthus retroflexus*, *Chaetochloa lutescens*, *Portulaca oleracea*, *Oenothera biennis*, *Rumex crispus*. These all made a good growth and were kept properly labeled.

The first of Dr. Beal's seeds to germinate was *Brassica nigra*. By May 7, 9 individuals of this species had come up and were identified. Having made good growth (2–3 inches), they were removed to allow room for other species. Possibly the second plant to appear was *Chenopodium album*, two plants of which had appeared by May 7, and were identified beyond question. Strangely, this species was not mentioned as being in the set. At about the same time two individuals of ragweed (*Ambrosia elatior*) were identified. By May 22, there were nineteen seedlings of *Oenothera biennis*, some of these being 3 inches high, nine seedlings of *Rumex crispus* about 4 inches high, one plant of *Portulaca oleracea* with the third pair of leaves developed, one plant of *Lepidium virginicum* with one pair of leaves besides the cotyledons developed, five plants of *Plantago major*, some up to 3 inches tall, one plant of *Amaranthus retroflexus*, and thirty-three plants of *Amaranthus* sp. (probably *A. graecizans*).

Among the twenty-three species which Dr. Beal buried, he included *Juglans nigra*, *Quercus rubra*, and *Thuja occidentalis*. He says that the first two were not put in the bottles, and I have found no evidence to show that the Thuja was put in with the smaller seeds. This leaves twenty species that were put in bottles, or a total of one thousand small seeds in each bottle. With this number of seeds in each bottle it can easily be seen that, unless great care was taken, a few seeds other than those intended might easily be put in the sets. This, I believe, might account for some of the irregularities observed. The amaranth seeds in this bottle were evidently meant to be those of *A. retroflexus*, but thirty-three out of the thirty-four specimens of amaranth seedlings observed seemed to be *A. graecizans*. The percentage of germination for the genus Amaranthus was therefore, in this case, 68 percent after an interval of 40 years. The irregularity in connection with *Chenopodium album* may be due to the great similarity between the seeds of Chenopodium and those of Amaranthus. Table 1 gives a summary of those seeds which germinated, with the number of individuals in each case and the percentage of germinations.

TABLE 1

Name of Plant	Number of Individuals	Percentage of Germination
Brassica nigra	9	18
Oenothera biennis	19	38
Rumex crispus	9	18
Portulaca oleracea	1	2
Plantago major	5	10
Amaranthus retroflexus	1	2
Amaranthus graecizans	33	66
Lepidium virginicum	1	2
Ambrosia elatior	2	4
(*Chenopodium album*)	2	

TABLE 2

Name of Species Tested	5th Yr.	10th Yr.	15th Yr.	20th Yr.	25th Yr.	30th Yr.	35th Yr.	40th Yr.
Amaranthus retroflexus	+	+	+	+	+	+	o	+
Ambrosia elatior	o	o	o	o	o	o	o	+
Brassica nigra	o	+	+	+	+	+	+	+
Bromus secalinus	o	o	o	o	o	o	o	o
Bursa bursa-pastoris	+	o	+	+	+	+	+	o
Erechtites hieracifolia	o	o	o	o	o	o	o	o
Euphorbia maculata	o	o	o	o	o	o	o	o
Lepidium virginicum	+	+	+	+	+	+	+	+
Lychnis githago	o	o	o	o	o	o	o	o
Anthemis cotula	+	+	+	o	+	o	?	o
Malva rotundifolia	+	o	o	+	o	o	o	o
Oenothera biennis	+	+	+	+	+	+	o	+
Plantago major	o	o	+	o	o	o	o	+
Polygonum hydropiper	o	+	+	+	+	possibly	o	o
Portulaca oleracea	o	+	+	+	+	o	o	+
Rumex crispus	+	?	+	+	+	+	+	+
Chaetochloa lutescens	+	+	+	o	+	+	o	o
Alsine media	+	+	+	+	+	+	o	o
Trifolium repens	o	o	o	o	o	o	o	o
Verbascum thapsus	+	?	+	+	o	o	+	o
(*Amaranthus graecizans*)								+
(*Chenopodium album*)								+

The total number of seedlings from one thousand seeds was eighty-two. The total percentage of germination was, therefore, at least 8.2 percent. It might be reasonably supposed that the real percentage of germination was even higher than this. Table 2 shows the results for forty years. From this it appears that Lepidium is the only species which has an unbroken record for every observation. From this latest test, Amaranthus certainly makes the best showing, with Oenothera second and *Brassica nigra* and *Rumex crispus* third. The facts that *Ambrosia elatior* had never been made to germinate before, and that *Plantago major* had germinated only once, would seem to indicate that conditions for germination were especially favorable. On the other hand, the question arises why *Bursa bursa-pastoris* and *Chaetochloa lutescens*, each of which have failed only once, failed to germinate. Table 2 gives the complete results of the experiment up to the spring of 1920.

Beal Botanical Laboratory,
East Lansing, Michigan.

NUTRIENT SOLUTIONS FOR WHEAT

W. E. Tottingham and E. J. Rankin[1]

(Received for publication September 28, 1921)

The following brief notes relate to collaborative work of the writers upon a project outlined by a special committee of the National Research Council (17). Publication in the present rather fragmentary condition is ventured in the hope that our observations may be of aid to others operating in the same field. Acknowledgment is hereby made of a grant from the research funds of the University of Wisconsin in support of this investigation.

Of the six possible three-salt combinations which can supply the six essential elements of a nutrient solution, exclusive of iron, as arranged in the plan for collaborators, Type VI was chosen for the present investigation. This type is composed of KH_2PO_4, $CaSO_4$, and $Mg(NO_3)_2$. Cultures of wheat were conducted in duplicate in the series of solutions of this type, following the prescribed plan. The results appear to justify no further statement here than that the agreement between duplicate cultures was generally very poor. It seemed probable that this condition was due in part to the very poor root development of the seedlings employed. The roots were short, and usually curved and discolored where they came in contact with the nutrient solution.

Shive's nutrient solution R5C2 (15) diluted to 0.1 the usual concentration is the medium prescribed for germination in this work. The pH of this nutrient solution, at the concentration commonly employed, is approximately 4.7 (16). It appeared possible that the poor root growth observed here was due partly to the acidity of this solution. With this possibility in mind, the following tests were undertaken. The seed used throughout was wheat of the Marquis variety, provided for collaborators by the committee in charge of the project. In tests other than the comparison of continuous with intermittent renewal of the solution, ferric citrate was used instead of ferric phosphate, as a source of iron.

pH in Relation to Germination

The best solution of the series tested by Livingston and Tottingham (9), R8C1, is unfavorable for the germination of wheat when continuously renewed at 0.001 the usual concentration of 1.75 atm. osmotic value. This solution is still decidedly acid, however, as indicated by the data of table 1, obtained by the use of Clark and Lubs' method (2):

---

[1] Published with permission of the director of the Wisconsin Experiment Station.

TABLE 1. *Change of pH Values of Solution R8C1, Type III, Three-Salt Solution*

Conc.	pH
Usual (1.75 atm.)	4.1
0.5 usual	4.3
0.1 usual	5.3
0.01 usual	5.4
0.001 usual	5.5

The same solution diluted to 0.1 its usual concentration was adjusted to pH values of 6.4 and 7.5 by the addition of KOH. When used as a medium for germination, including preliminary soaking of the seeds and suspension on netting upon the flowing solution, this nutrient solution at pH 6.4 gave root systems but little superior to those obtained at pH 5.3. At pH 7.5, however, the roots elongated in a satisfactory manner, so that the seedlings were employed in a test described later.

These results hardly agree with those of Hixon (4), who found pH values both below and above 6.0 favorable for elongation of roots in the germination of wheat. It should be noted, however, that his solutions were not renewed. Salter and McIlvaine (14), employing the method of intermittent renewal of nutrient solutions, reach conclusions directly opposed to ours, namely:

> Germination of the seed was found less sensitive to an acid reaction . . . . than was the subsequent growth of the seedling.

Their criterion of growth, however, was green weight of seedlings. Their photographs of wheat seedlings show best root development at pH 7.7. Inasmuch as wheat grows well at much lower pH values than 6.4, as shown by Duggar (3), and appears to be independent of hydrogen-ion concentration in yield of dry matter, at least in intermittently renewed solutions, as observed by Meir and Halsted (12) and by McCall and Haig (10), it seems possible that its peculiar sensitiveness to pH values during germination may be related to some factor inherent in the seed, such as the isoelectric points of its proteins.

The adjustment here developed seems to offer a means of providing more uniform seedlings for different collaborators, by use of a common solution for germination, should it seem advisable to continue such practice.

COMPARISON OF CONTINUOUS WITH INTERMITTENT RENEWAL OF THE NUTRIENT SOLUTION

One of the fundamental desiderata of investigations with nutrient solutions is the avoidance of disturbing effects of the absorbing plant upon the concentrations and proportions of nutrients available to it. This point was stressed long ago by Nobbe (13) and has been re-emphasized by Hoagland (6).

For the present purpose Shive's solution R5C2 was employed. The seedlings were reared in flowing tap water tempered to about 25 degrees C. This water is drawn from Lake Mendota and has the following composition in p. p. m.:[2] K 2.2, Ca 19.8, Mg 21.6, P undetermined, S 5.0, N 1.1, pH approximately 7.5. It supports excellent growth of wheat seedlings, especially as regards the root systems. Three duplicate cultures of 5 plants each were mounted in wide-mouthed jars of about 480 cc. capacity (Mason pint jars) through which the nutrient solution flowed continuously at the rate of about 4 liters per jar daily. The nutrient solutions of three other similar cultures were renewed every third day. Finally, three duplicate cultures were grown in jars of about 240-cc. capacity and received fresh solution every third day. These cultures grew from February 14 to March 1, 1921. No climatic records were taken in the greenhouse during this period. The data of yields are assembled in table 2.

TABLE 2. *Influence on Growth of Proportion of Plants to Nutrient Solution*

Form of Renewal	Capacity of Culture Vessel	Average Length		Average Dry Weight	
		Roots	Tops	Roots	Tops
	Cc.	Mm.	Mm.	Mg.	Mg.
Continuous	480	121 (5)*	Not measured	114 (11)	232 (13)
3-day	480	150 (8)		130 (16)	338 (23)
3-day	240	121 (5)		140 (12)	353 (45)

* Average departure from mean.

On the basis of dry weight of plants produced, the method of intermittent renewal was superior to that of continuous replacement. These results are not in agreement with those of Trelease and Free (20), but the rates of continuous renewal, and probably also the climatic complex, differed in the two tests.

Conditions generally associated with injury from acid, such as darkening and withering of leaf tips, were apparent in all the cultures at the time of harvesting. They appeared earliest and most severely, however, in the cultures whose solutions were continuously renewed. We interpret this difference as due to the ability of the plants to reduce the hydrogen-ion concentration when the solutions were renewed at intervals, as demonstrated by Hoagland (7, p. 101), Toole and Tottingham (19) and Duggar (3, pp. 11, 15, 19). It may be noted that growth was most uniform in the cultures whose nutrient supply was continuously renewed. These results support the assumed importance of continuous renewal of nutrient solutions, or equivalent treatment, so that the plant may be subjected continuously to a constant composition of the nutrient medium.

---

[2] Compiled from data of the Wisconsin Geological and Natural History Survey and of the State Hygienic Laboratory.

Comparison of Different Concentrations and Renewal Rates of Solutions

Wheat seedlings reared on flowing tap water were mounted in groups of 5 in each 480-cc. jar. Three jars received Livingston and Tottingham's solution R8C1 diluted to 0.1 the usual concentration and renewed continuously, approximately at the rate of 2 liters per jar in 24 hours. A second group of three jars received 0.01 the usual concentration of the same solution at the rate of 4 liters per jar in 24 hours. Finally, a third set of jars received the solution at 0.001 the usual concentration at the rate of 8 liters per jar in 24 hours. It will be noted that the rates of renewal were not increased in proportion to the dilution of the nutrient solution. The proportions used were dictated largely by practical considerations of time and apparatus available.

The young plants were grown under these conditions from May 3 to May 16, 1921. During this period the atmospheric conditions of the greenhouse, as measured by atmometers (Livingston, 8), were as follows:

Mean daily evaporation from standard spherical white atmometer, 20.4 cc.
Mean daily evaporation from standard spherical black atmometer, 25.6 cc.
Ratio of black to white instrument, 1.25.

The cultures at 0.001 the usual concentration were discontinued on May 7, when the plants showed decided signs of withering, with little growth. At the time of discontinuing the series, the plants grown in 0.01 the usual concentration of solution were decidedly withered and had apparently ceased to grow, while the leaves of cultures grown in 0.1 the usual concentration of solution were beginning to turn yellow. The yields are presented in table 3.

Table 3. *Influence upon Growth of Concentration and Rate of Renewal of the Nutrient Solution*

Concentration of Solution	Rate of Supply	Average Length		Average Dry Weight	
		Tops	Roots	Tops	Roots
		Mm.	Mm.	Mg.	Mg.
0.1 usual	2 l. in 24 hr.	225 (38)*	84 (3)	201 (9)	117 (10)
0.01 "	4 " " " "	144 (19)	72 (3)	92 (27)	70 (3)
0.001 "	8 " " " "	Practically no growth			

* Average departure from mean.

From these data it appears that, for these rates of renewal, 0.01 the usual concentration of this nutrient solution was not nearly sufficient for maximum growth. The limits of practicability in rate of supply and degree of dilution of the solution for maximum growth are important points for further investigation.

Comparison of Different pH Values with Continuously Renewed Solutions

On the basis of the results of the preceding experiment, 0.1 the usual concentration of Livingston and Tottingham's solution R8C1 was chosen for this test. Two cultures were arranged at each of three degrees of acidity as follows: 5.3 pH, 6.4 pH, 7.5 pH. The pH values were adjusted by adding KOH with use of the appropriate indicators used by Clark and Lubs. For this purpose, 0.9 cc. of *N*/2 KOH per liter of diluted nutrient solution was required for the highest pH value and 0.5 cc. for the intermediate one. Seedlings reared in flowing tap water were grown in these solutions of different pH values, continuously renewed at approximately the rate of 2 liters per jar per 24 hours. The growth period extended from May 20 to June 7. During this time the atmometer measurements taken adjacent to the cultures were as follows:

Mean daily evaporation from standard spherical white atmometer, 19.1 cc.
Mean daily evaporation from standard spherical black atmometer, 25.1 cc.
Ratio of black to white instrument, 1.31.

The data of yields appear in table 4.

Table 4

pH Value of Solution	Average Length		Average Dry Weight	
	Tops	Roots	Tops	Roots
	Mm.	Mm.	Mg.	Mg.
5.3	182 (18)*	106 (6)	229 (9)	108 (5)
6.4	258 (45)	245 (33)	367 (76)	198 (17)
7.5	295 (58)	300 (75)	512 (218)	230 (35)
Second Test				
6.4	178 (3)	175 (25)	170 (9)	96 (3)
7.5	188 (0)	213 (13)	141 (5)	75 (3)

* Average departure from mean.

At the end of the test the plants grown at pH 5.3 had short root systems, especially as regards secondary roots, and the leaf tips were withered. Both of these conditions are characteristic of acid injury. The growth response at the two higher pH values was irregular. Both groups of plants at pH 6.4 were superior to those grown at pH 5.3, but one culture at the former pH value was much superior to the other in appearance of roots and tops. At pH 7.5 one culture was but little superior to the poorer one at pH 6.4, while the other gave profuse elongation of roots and development of tops.

In view of these discrepancies, the comparison of pH 6.4 and 7.5 was repeated. For this purpose seedlings were available which had been reared in Livingston and Tottingham's solution R8C1 at 0.1 the usual concentration and adjusted to pH 7.5. The plants grew in the solutions from June 7 to June 14. During this period the climatic measurements were as follows:

Mean daily evaporation from standard spherical white atmometer, 16.6 cc.
Mean daily evaporation from standard spherical black atmometer, 23.3 cc.
Ratio of black to white instrument, 1.40.

The growth data of this test also appear in table 4.

When dismounted, the appearance of the tops grown in the solution having a pH value of 7.5 was superior to that of plants grown at pH 6.4. The latter were developing yellow color in the leaves. The combined tests indicate that pH 7.5 excels pH 6.4 on the basis of elongation of the plants, but that the lower pH leads to greater production of dry matter. The latter result compares favorably with previous results from solutions intermittently renewed, which also have shown a favorable effect of slight acidity of the culture solution as measured by production of dry matter. As suggested by Meir and Halsted (12), however, solutions of relatively high hydrogen-ion concentration may be efficient on account of their correspondingly high phosphate content and resulting buffer capacity, rather than because of acidity *per se*. They may also favor availability of iron, as suggested by McCall and Haig (11).

Conclusions

The optimal nutrient conditions, as regards pH values, appear to differ as between the germination phase and the later growth of wheat. Hydrogen-ion concentrations which are endured by the wheat plant in intermittently renewed solutions become unendurable when the solution is continuously renewed. Certain pH values which restrict the elongation of stem and root in wheat appear to favor the production of dry matter in these organs.

The above noted relations enhance the importance of specification of the standards by which nutrient efficiency is to be measured. They also suggest the advisability of employing the method of continuous renewal of the nutrient solution, or equivalent procedure, for more rigid control of experimental conditions. Obviously, in its relation to agricultural practice, this suggestion involves consideration of the extent to which field crops modify the composition of the soil solution. Inasmuch as changes of concentration of the water-soluble constituents have been found to follow cropping of the soil (Stewart, 18; Burd, 1), there appears to be a considerable degree of practicability in the original plan of intermittent renewal of nutrient solutions.

Department of Agricultural Chemistry,
University of Wisconsin

BIBLIOGRAPHY

1. **Burd, J. S.** Rate of absorption of soil constituents at successive stages of plant growth. Jour. Agr. Res. **18**: 51–72. 1919.
2. **Clark, W. M., and Lubs, H. W.** Colorimetric determination of hydrogen ion concentration and its applications in bacteriology. Jour. Bact. **2**: 1–34, 109–136, 191–236. 1917.
3. **Duggar, B. M.** Hydrogen ion concentration and the composition of nutrient solutions in relation to the growth of seed plants. Ann. Mo. Bot. Gard. **7**: 1–49. 1920.
4. **Hixon, R. M.** The effect of the reaction of a nutritive solution on germination and the first stages of growth. Medd. K. Vetenskapsakad. Nobelinstitut **4**: 1–28. 1920.
5. **Hoagland, D. R.** The freezing point method as an index of variations in the soil solution due to season and crop growth. Jour. Agr. Res. **12**: 369–395. 1918.
6. ——. Note on the technique of solution culture experiments with plants. Science n. ser. **49**: 360–362. 1919.
7. ——. Relation of the concentration and reaction of the nutrient medium to the growth and absorption of the plant. Jour. Agr. Res. **18**: 73–117. 1919.
8. **Livingston, B. E.** Atmometry and the porous cup atmometer. Plant World **18**: 21–30, 51–74, 95–111, 143–149. 1915.
9. ——**, and Tottingham, W. E.** A new three-salt nutrient solution for plant cultures. Amer. Jour. Bot. **5**: 337–346. 1918.
10. **McCall, A. G., and Haig, J. R.** The hydrogen ion concentration of certain three-salt nutrient solutions for plants. Soil Sci. **10**: 481–485. 1920.
11. ——. The relation of hydrogen ion concentration of nutrient solutions to growth and chlorosis of wheat plants. Soil Sci. **12**: 69–77. 1921.
12. **Meir, H. F., and Halsted, C. E.** Hydrogen ion concentration relations in a three-salt solution. Soil Sci. **11**: 325–350. 1921.
13. **Nobbe, F.** Die Zuchtung der Landpflanzen in Wasser betreffend. Landw. Versuchsst. **7**: 68–73. 1865.
14. **Salter, R. M., and McIlvaine, T. C.** Effect of reaction of solution on germination of seed and growth of seedlings. Jour. Agr. Res. **19**: 73–95. 1920.
15. **Shive, J. W.** A study of physiological balance in nutrient media. Physiol. Res. **1**: 327–397. 1915.
16. ——. The influence of sand upon the concentration and reaction of a nutrient solution for plants. Soil Sci. **9**: 169–179. 1920.
17. Special Committee of the Division of Biology and Agriculture, National Research Council. Plan for coöperative research on the salt requirements of representative agricultural plants. Pp. 29. Ed. by B. E. Livingston. Baltimore, 1918. Revised 1919.
18. **Stewart, G. R.** Effect of season and crop growth in modifying the soil extract. Jour. Agr. Res. **12**: 311–368. 1918.
19. **Toole, E. H., and Tottingham, W. E.** The influence of certain added solids upon the composition and efficiency of Knop's nutrient solution. Amer. Jour. Bot. **5**: 452–461. 1918.
20. **Trelease, S. F., and Free, E. E.** The effect of renewal of culture solutions on the growth of young wheat plants in water-cultures. Johns Hopkins Univ. Circ. n. ser. **3**: 227, 228. 1917.

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1922

President: Henry C. Cowles
University of Chicago

Vice-President: Margaret C. Ferguson
Wellesley College

Secretary: I. F. Lewis
University of Virginia, University, Va.

Treasurer: Irving W. Bailey
Bussey Institution, Harvard University

Councilors:

N. L. Britton
New York Botanical Garden

J. C. Arthur
Purdue University

C. E. Allen
University of Wisconsin

Any one actively interested in botanical work is eligible for membership Candidates for membership must be recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including one subscription to the American Journal of Botany. Candidates proposed for membership, and approved by the Council, may receive one copy of the Journal at $4.00 per year pending their election at the winter meeting of the Society.

Do Our Greenhouses Really Differ So Much From Others

Outwardly most folks are mostly alike.

So are most greenhouses.

But inwardly—that's quite a different matter.

Just as the way a man gives attention to the little things—tells you the kind of a man he is; just so the care and particularity given to the little details in a greenhouse, tells you the kind of a house it is.

Some owners have accused us of being rather fussy about our work, intimating that we could cut corners and get the work done quicker.

And they are right.

But you'll agree that we are also right in refusing to do it.

That then might be one of the differences in which our greenhouses differ.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

PHILADELPHIA
133 So. 12th St.

Vol. IX. JUNE, 1922 No. 6

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

CONTENTS

Comparative studies on respiration XXI. Acid formation and decreased production of CO_2 due to ethyl alcohol . . . . MARIAN IRWIN AND MARGARET WEINSTEIN 277
A new species of Spirogyra with unusual arrangement of the chromatophores. MABEL L. MERRIMAN 283
The male receptacle and antheridium of Reboulia hemisphaerica . . . . A. W. DUPLER 285
The meiotic cytokinesis of Nelumbo . . . . CLIFFORD H. FARR 296
Comparative studies on respiration XXII. The effect of lactic acid on the respiration of wheat . . . . EDITH PHILIP SMITH 307
The effect of transpiration on the absorption of salts by plants . . . . WALTER C. MUENSCHER 311
Prairie inclusions in the deciduous forest climax . . . . HERBERT C. HANSON 330

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT PRINCE AND LEMON STS., LANCASTER, PA.
LONDON AGENTS
WHELDON AND WESLEY, LTD.
28 ESSEX STREET, STRAND, LONDON, W. C. 2

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania,
under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
The Thompson Institute For Plant Research, Inc.

H. H. BARTLETT,
University of Michigan

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ARTHUR H. GRAVES,
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

I. F. LEWIS,
University of Virginia

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Postage to Canada, 20 cents; to other foreign countries (except Mexico and Cuba) 40 cents a volume on annual subscriptions. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume, post free.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or The New Era Printing Co., Inc., Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. IX JUNE, 1922 NO. 6

COMPARATIVE STUDIES ON RESPIRATION XXI.

ACID FORMATION AND DECREASED PRODUCTION OF CO_2 DUE TO ETHYL ALCOHOL

MARIAN IRWIN AND MARGARET WEINSTEIN

(Received for publication October 1, 1921)

The purpose of the present investigation is to determine the effect of ethyl alcohol on the production of carbon dioxide by seedlings of the radish (Early French Breakfast).

The seeds were allowed to germinate on moist filter paper until the caulicles were about 5 mm. long: they were then used for the experiments. The apparatus used for the determination of carbon dioxide production is described in a previous paper.[1] It is important first to test the apparatus for leakage and to allow the air to pass through the tube containing the lumps of sodium hydroxide until the whole apparatus is practically free from carbon dioxide. The tube is then disconnected and the organisms are placed in it. It is important that a check experiment, without the seedlings, should be made to ascertain whether the entrance of the laboratory air on disconnecting the tube introduces any error. After the seedlings are placed in the tube and the whole apparatus is again connected, the air is again pumped through a tube containing sodium hydroxide for three minutes to absorb the carbon dioxide which is introduced when the tube is disconnected.

The period during which the liquid containing the indicator changes from pH 8 to pH 7.6 is recorded by a stop watch, and the reciprocal of this time is taken as the rate of production of carbon dioxide by the seedlings.

The color of the indicator is affected by alcohol, which makes it necessary to correct the readings by calibrating the indicator solutions which have taken up alcohol vapor by adding to them measured amounts of carbon dioxide. This must be done for each concentration of alcohol.

In order to ascertain the normal rate of respiration, seedlings are placed in tap water and readings are taken every ten minutes for three hours. Curve *A*, figure 1, shows that a series of readings of this sort agree, and it is evident that the experimental errors are not large. An average of the

---

[1] Irwin, M. Jour. Gen. Physiol. 3 : 203–206. 1920.

[The **Journal** for May (9: 213–276) was issued June 21, 1922]

whole series is taken, and this is considered to be 100 percent; with this average as the standard, the relative rate of production of carbon dioxide under the influence of alcohol is calculated.

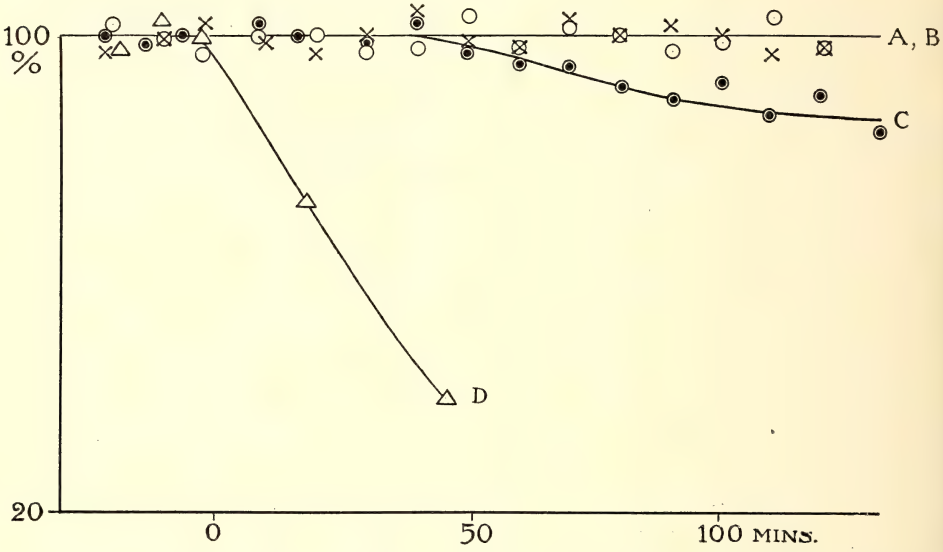


Fig. 1. Curves showing the effect of ethyl alcohol on the production of carbon dioxide by radish seedlings. To the left of the point O on the abscissa the curve shows the rate of respiration in tap water; to the right of that point, the rate under the influence of alcohol, except in curve *A* which represents the rate in tap water (without exposure to alcohol). Curve *B* shows the rate in 1*M* alcohol, curve *C* in 2*M* alcohol and curve *D* in 4*M* alcohol. Each point represents an average of 12 experiments. Probable error of the mean is less than 3 percent of the mean.

Before exposing the seedlings to a solution of alcohol, control readings are made with the seedlings in 3 cc. of tap water. The seedlings are then taken out of the apparatus and kept in a large dish of water. A bottle with a capacity of 250 cc., filled with a solution of alcohol, is fitted to the portion of the apparatus to which the tube which is to contain organisms is usually fitted. The liquid containing the indicator is now replaced by the same amount of liquid of the same concentration of alcohol as that contained in the larger bottle and having the same concentration of indicator. The whole apparatus is thus saturated with the alcohol vapor in equilibrium with the solution which is to be used for the experiment. Then 3 cc. of alcohol of the same concentration is placed in the tube and the air is again passed through the U-tube containing sodium hydroxide until the whole apparatus is again free of carbon dioxide. The organisms are now placed in the tube and the air is passed through the sodium hydroxide for three minutes, after which the reading is made in the same manner as in the control experiment. The relative rate of respiration of the seedlings in alcohol is calculated on the basis of the average of the control experiment, which was made immediately before the experiment with the alcohol.

With $1M$ alcohol the production of carbon dioxide by seedlings remains unchanged for the first hour, as shown by figure 1, curve *B*. With $2M$ alcohol there is a slight decrease during the first hour (fig. 1, curve *C*). With $4M$ solutions, a great decrease takes place in about twenty minutes (fig. 1, curve *D*). This change is irreversible.

The fact that death is often accompanied by an increase in the production of CO_2 might lead to the suspicion that such an increase also occurs in the case of alcohol.

With the apparatus used for the present investigation, the first reading cannot be taken within ten or fifteen minutes after the immersion of the seedlings in the test solution. For this reason it might be supposed that an increase followed by a decrease may have taken place during the first fifteen minutes of the experiment. If an increase really occurred then, it seems probable (on the basis of other experiments) that it would persist for a time after removal from the alcohol. In order to test this, the seedlings were put in $4M$ alcohol, removed and washed for half a minute in running water, and then put into the apparatus with 3 cc. of tap water, after which the readings were made. Exposure of radish seedlings to the alcohol for three minutes brought about no change in the rate of respiration, while there was a slight decrease after an exposure of five minutes, and a greater decrease after exposures of seven and ten minutes, as shown in figure 2, curves *B*,

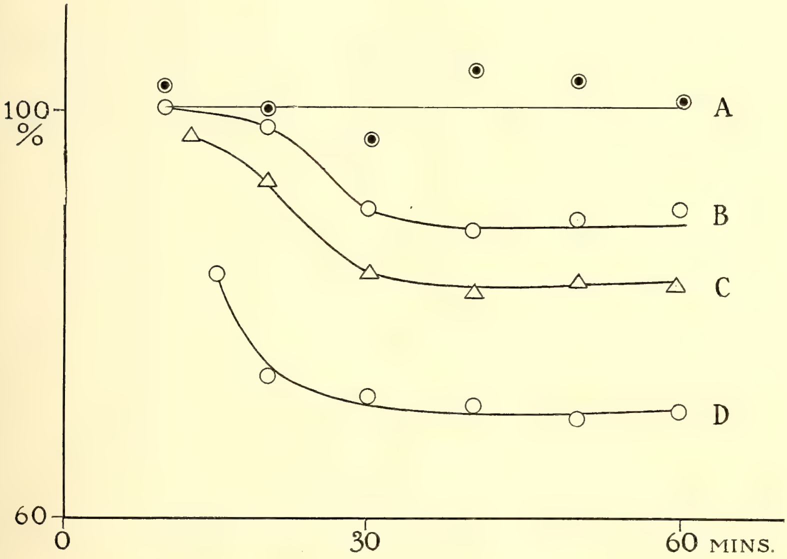


FIG. 2. Curves showing the effects of $4M$ ethyl alcohol on the production of carbon dioxide by radish seedlings. Curve *A* shows the rate of respiration in tap water (without exposure to alcohol). Curve *B* shows the rate after the seedlings have been exposed to the alcohol for 5 minutes and then replaced in tap water; curve *C*, exposed for 7 minutes; curve *D*, for 10 minutes. Each point represents the average of 12 experiments. Probable error of the mean is less than 4 percent of the mean.

C, and *D*. These experiments seem to indicate that alcohol does not produce an initial increase in the rate of production of carbon dioxide.

After experimentation the seeds were allowed to grow on wet filter paper in a moist chamber in sunlight. If they had been exposed for ten minutes to $4M$ alcohol, there was no subsequent growth. After an exposure of five minutes there was slight growth of the root tip and considerable (though retarded) growth of the stem. After an exposure of three minutes the retardation of growth was very slight.

It is difficult to make any comparisons between growth and the production of carbon dioxide for the reason that the susceptibility of the different parts of the seedlings varies. The root tip is most sensitive, so that a short exposure may influence its growth considerably, but it represents only a small portion of the seedling, so that its injury may not make much difference in the production of carbon dioxide if the remaining, less susceptible portion is left unaffected. A great decrease in the production of carbon dioxide takes place only when the entire seedling is affected to such an extent that there is no subsequent growth.

When the seedlings are exposed to $4M$ solutions of alcohol for about five minutes, formation of acids other than carbonic takes place. This may be detected by the following method. Twenty seedlings were put into a glass-stoppered Pyrex tube with 3 cc. of tap water (containing the same amount of indicator as in the standard tube) for twenty minutes; 2 cc. of this liquid were then put into the indicator tube, and air free from carbon dioxide was passed into the tube just as in the previous experiments. The time required to bring the liquid to pH 7.6 by thus driving carbon dioxide out of the liquid was about three minutes. The same seedlings were now placed in a glass-stoppered tube containing a $4M$ solution of alcohol, washed for one half minute in running water and placed in 3 cc. of tap water for twenty minutes. 2 cc. of this liquid were then placed in the indicator tube, and the air free from carbon dioxide was passed through it to drive out the carbon dioxide. After three minutes' exposure of the seedlings to $4M$ alcohol, the time recorded was about the same as in the control. This indicates that there is no acid other than carbonic in the liquid.[2] But when the seedlings had been exposed for five minutes to the same concentration of alcohol the change was very much slower; in many cases the change did not take place within ten minutes. After ten minutes there was no change within twenty minutes. This proves that acids, other than carbonic, must be present in the liquid. The slowness of the change back to pH 7.6 cannot be due to excess of carbon dioxide, since the previous experiments show that less carbon dioxide is produced in $4M$ alcohol.

The question arises whether the presence of such acids is due to the extraction by alcohol of acids already present before the experiment was

[2] When other volatile acids are absorbed by water, it is difficult to drive the acid out of the water by a current of air and bring the water back to neutrality.

begun, or whether it is due to the actual formation of such acids as a direct effect of the alcohol on the cells. It was found that when seedlings were killed in boiling water the presence of the acids could be demonstrated. Since enzymes are generally destroyed at this temperature, this experiment might seem to show that the acids were already present in the uninjured cells. However, in the case of oxidizing enzymes it has been shown that it is very difficult to destroy the enzymes by this method rapidly enough to prevent the oxidation completely. This is readily demonstrated in the Indian pipe (*Monotropa uniflora*), in which case it is difficult to destroy the enzymes rapidly enough by immersing the plant in boiling water to prevent the appearance of the black pigment.

If the acids are normally present, it would be expected that they could be detected by killing the seedlings by liquid air. But when seedlings were exposed to liquid air and then crushed and extracted with cold water, there was no evidence of the presence of these acids.

Seedlings were allowed to dry for one month on filter paper at room temperature and were then crushed and extracted with cold water, and the acids were found. The production of carbon dioxide by the dried seedlings, after they were replaced in water, was found to be about one half the normal. Growth occurred when the seedlings were again soaked in water, but it was somewhat retarded.

It is difficult to draw definite conclusions from these experiments, but it is possible that ethyl alcohol accelerates the decomposition of certain substances, forming an excess of intermediate products in the form of organic acids, until the concentration of the hydrogen ion (or of some other substance) increases to such an extent that any further decomposition into carbon dioxide and water is partly inhibited. The nature of the organic acids has not been determined, but further investigations may throw light on this point.

The question may be raised whether the production of acids other than carbonic is due to the oxidation of the alcohol. Oxidation[3] of alcohol by higher plants has been reported. But in the experiments described above oxidation of ethyl alcohol does not seem to be the chief cause of the formation of organic acids, because, as it was already shown, organic acids were formed when the seedlings were put in boiling water without having been treated with alcohol.

It may be of interest to note the extreme complexity of the problems of respiration as indicated by the difference in the behavior of the radish seedlings in ethyl alcohol, as contrasted with the behavior of Salvia in ether.[4] In the case of radish seedlings production of carbon dioxide is decreased and the acidity of the tissue apparently increases, while in the case of Salvia production of carbon dioxide is increased and the alkalinity increases.

Loeb and Wasteneys[5] showed that ethyl alcohol decreases the consumption of oxygen by *Strongylocentrotus purpuratus*, but that this is not sufficient to account for the narcotic effect on the basis of decrease of oxygen consumption (though a decrease of about sixteen percent of the normal is brought about). They point out that this is in disagreement with Verworn's theory. The experiments of the writers are in harmony with this conclusion.

---

[3] Zaleski, W. Biochem. Zeitschr. 69 : 283–289. 1915.

[4] Irwin, M. Jour. Gen. Physiol. 1 : 399–403. 1919.

[5] Loeb, J., and Wasteneys, H. Jour. Biol. Chem. 14 : 517–523. 1913.

SUMMARY

Ethyl alcohol decreases the production of carbon dioxide by radish seedlings and at the same time brings about the formation of organic acids.

Laboratory of Plant Physiology,
Harvard University

A NEW SPECIES OF SPIROGYRA WITH UNUSUAL ARRANGEMENT OF THE CHROMATOPHORES

Mabel L. Merriman

(Received for publication October 24, 1921)

In November, 1920, collections of algae in Van Cortlandt Park, New York City, revealed a large form of Spirogyra with a peculiar disposition of its chromatophores. The collections made at various times during the next two months showed the alga freely conjugating and growing in fairly pure cultures. It was noted that, whereas in the non-conjugating filaments at this time the chromatophores were more or less spirally arranged, no case was found of a vegetative cell adjoining conjugating cells where the chromatophores were other than parallel in their position. At the time it was thought that the plant might be a variety of *S. crassa*. Subsequent collections in May and June revealed *S. crassa* growing in abundance with the new form, both abundantly conjugating, and when seen side by side exhibiting characters conspicuously distinct. To date no case has been found of the hybridization of the two forms.

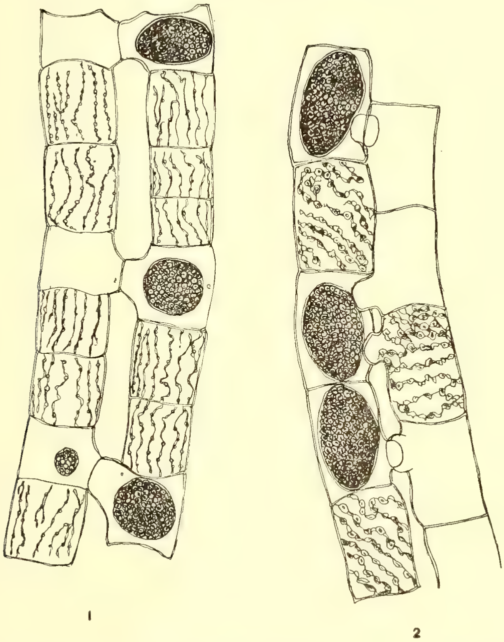


Fig. 1. Conjugating filament, with zygospores, of *S. rectispira*, from material collected June, 1921. × 140. Fig. 2. Conjugating filament, with zygospores, of *S. crassa* growing with *S. rectispira*.

Figures 1 and 2 are of examples taken from the material collected in June. This material shows a much greater uniformity in the arrangement of the chromatophores, most of them being parallel irrespective of their location in conjugating filaments. Figure 3 is of a conjugating form from the material collected in November, 1920. In this by no means exceptional case, the chromatophores in the course of a few cells may be seen to change from regular to loose spirals, and finally to straight and discontinuous bands as the vegetative cells approximate in position the fertile cells of the filaments. Such successive relaxation in the bands is worthy of further investigation, for in this behavior may be found a clue to the understanding of some of the physiological changes that the cells undergo in their transition from the vegetative to the reproductive state.

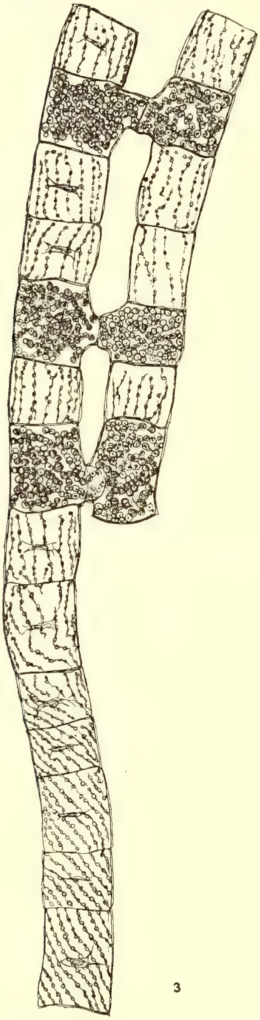


FIG. 3. *S. rectispira* collected in November, 1920, showing changes in position of chromatophores in cells in a linear series.

The following is a description of this new form which may be appropriately designated as *S. rectispira.*

Spirogyra rectispira. Diameter of vegetative filaments 150–160μ, cells ½ to 2 times longer than diameter, chromatophores 6–11; chromatophores straight or somewhat sinuous, sparingly branched or discontinuous; in some filaments, particularly when not conjugating, all gradations to be seen from straight chromatophores in cells adjoining conjugating cells to spirals making ½ to 1 turn in the cell. Fertile cells not or only slightly inflated, zygospores 140–108μ, orbicular or subglobose, not completely filling the cells.

This species ranks with *S. crassa* in the diameter of its filaments as the largest of the genus. It is distinguished from *S. crassa* by its much smaller zygospores and by the parallel arrangement of the chromatophores in vegetative cells of conjugating threads. The pyrenoids with their starch accretions are at all times much smaller than those of *S. crassa*, and the chromatophores are much narrower. At the beginning of conjugation all the bodies making up the cell contents become greatly increased in size, filling the cells, the latter before the blending of the chromatophores of the two sexual cells being a brilliant green in contrast to the paucity of chlorophyll in the adjoining vegetative cells.

HUNTER COLLEGE,
NEW YORK CITY

THE MALE RECEPTACLE AND ANTHERIDIUM OF *REBOULIA HEMISPHAERICA*

A. W. Dupler

(Received for publication October 28, 1921)

The genus Reboulia has received less attention from morphologists than certain other forms of the Marchantiales, although recent papers by C. and R. Douin (10), Woodburn (29), and Haupt (16, 17) give evidence of a present interest in the form. The writer has shared in this interest and for some time has had in progress a study of *R. hemisphaerica* (L.) Raddi with a view to securing as complete an account of its morphology as a large collection of material would afford. The results of the writer's study of the origin and structure of the air chambers have been recently presented (11). Haupt's account is the only critical study of the sex organs to date, and should receive due credit as such. The following account of the antheridial structures confirms the work of Haupt in some features and supplements it in others.

The material used in this study was for the most part collected from the lower margins of a north-exposed talus slope at the base of a steep shale cliff, near Huntingdon, Pennsylvania, at intervals from 1912 to 1920. A second group of material was secured from near Mount Carroll, Illinois, in January, 1916, and kept in good growing condition in the botanical greenhouse of the University of Chicago. The last collection from this material was made in August, 1916. Under field conditions antheridia develop during the latter part of the summer, especially in August. In the greenhouse cultures antheridia were forming as early as April, although the August collections also showed antheridial receptacles in early stages of development.

HISTORICAL

The early study of Reboulia was concerned with its taxonomic features. Micheli (24) gave good figures of the male disc, describing the plant as "*Hepatica media capitulo hemispherico.*" Bischoff (1) noted the median or terminal position of the flat receptacle and the erect antheridia imbedded within its tissue. Hofmeister (18) observed the mature antheridium, claiming the tabular wall to be supplanted toward maturity by a membranous sac. He cites and figures the male receptacle directly behind the female receptacle. His suggestion that it is "probable that these [antheridial] cushions may be weakly developed shoots" is of interest. Leitgeb (21, 22) regarded the receptacle as a dorsal outgrowth, not involving the thallus apex in its formation, but in cases when the apex is permanently checked the receptacle appears terminal. He found that the thallus usually renews growth and may produce one or more successive discs. Leitgeb agrees with Nees ab Esenbeck (25) that the female receptacle is near the male but on a different branch, the thallus forking just before sex-organ formation and the one fork producing a male, the other a female receptacle, although both forks may produce the same sex organs. He gives no case in which both receptacles are on the same branch.

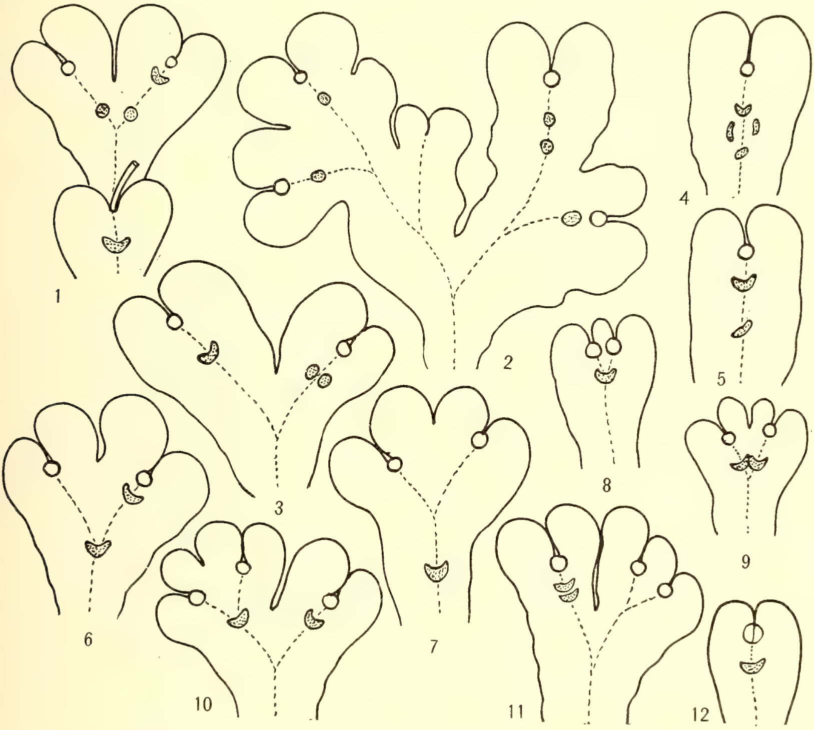
Cavers describes features of Reboulia in several of his papers, giving the only general account of its morphology which we have. He noted (4) the explosive discharge of the sperms, as high as 5 cm. Other phases of Cavers' work will be cited below.

C. and R. Douin (10) review the literature on the "inflorescence" of Reboulia, especially as it relates to the sexual nature of the plant. They credit Boulay (2) as the only observer previous to themselves who has noted marginal male receptacles. They define the antheridial disc as occurring at the end of a ventral or "subfloral" branch, while the female receptacle appears at the tip of an apical branch derived from the former and seeming to be a prolongation of it. They regard this as a distinctive character of Reboulia—"aucun autre genre de Marchantiées ne possède ce caractère." Haupt (16) describes the early antheridium in detail.

The Male Receptacle

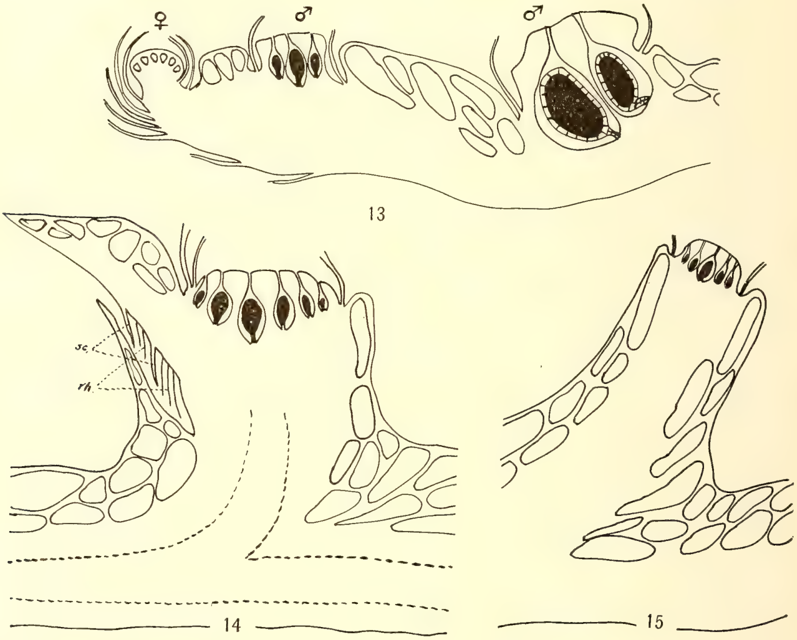
Position. The thallus arises as a ventral branch of the previous season's growth, growing out laterally from the midrib or, more commonly, longitudinally from beneath the base of the stalk of the female receptacle, giving a jointed aspect to a two-year plant (fig. 1). The male receptacle occupies a median dorsal position a short distance behind the female receptacle which terminates the branch. Two successive male receptacles are common (figs. 1, 2). Occasionally two discs may be lateral to one another (fig. 3), and rarely a cluster of several discs may be formed (fig. 4). The shoot may form both male and female receptacles without forking (fig. 5), although usually the primary shoot forks from one to three times during a season (fig. 2). The male receptacle may arise before or after the time of forking (compare figs. 1–12). The Douins (10) used the position of the male receptacle as one of the characters in separating from the polymorphic *R. hemisphaerica* of Stephani (26) two new species, *R. occidentalis* and *R. Charrieri,* in both of which the male receptacles are marginal instead of median as in *R. hemisphaerica.* Haupt (16) found a few rare cases of marginal receptacles in this form. The writer has not found a single case in hundreds of plants examined. Haupt thinks there is little justification for the new species. The Douins' claims were based on a careful study of plants grown under similar cultural conditions and can not be set aside summarily.

Form. In both the proposed new species of Douin the "primary disc" is said to arise as a terminal lunate structure which becomes divided by the growth of the "apical branch" into two circular "secondary discs," these being pushed apart by the widening of the thallus between them and finally coming to occupy a marginal position. The writer has not found that the later growth of the thallus affects in any way the form of the receptacle, even the lunate disc not being at all correlated with the forking of the thallus (figs. 1, 5, 9, 11). Both oval and lunate discs may occur on the same branch (fig. 1), or two oval (fig. 2) or two lunate discs may succeed one another (fig. 11).



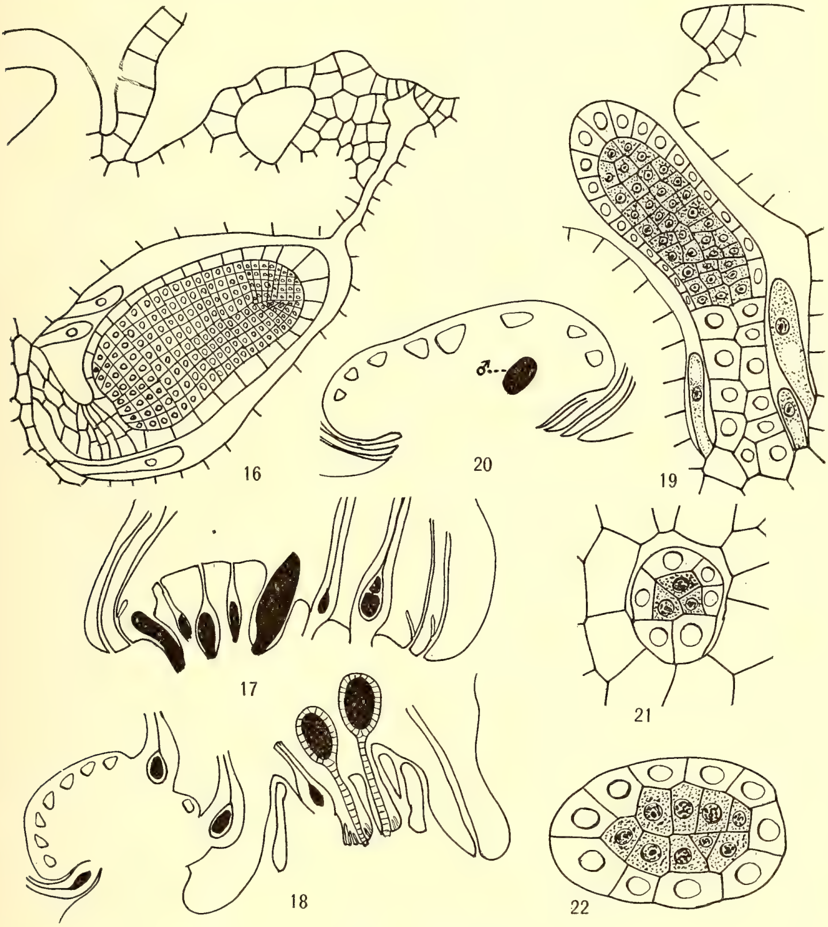
FIGS. 1–12. Habit of the plant of *Reboulia hemisphaerica,* showing the relation of female and male receptacles to one another and to the branching of the thallus. Male receptacles dotted. The dotted line indicates the midrib axis. Figure 1 shows a portion of the previous season's thallus with the base of the stalk of the old female receptacle. × 1.5.

Structure. The tissue of the young male disc is rather compact, but with the development of the antheridia the growth of the disc tissue exceeds that of the sex organs which thus become deeply imbedded in deep chambers opening to the exterior by narrow canals and simple air pores (fig. 16). Small air chambers develop between the canals. These chambers have simple pores, so far as found by both Haupt and the writer. Cavers (6) claims small barrel-type openings to occur occasionally. The disc is usually surrounded by a groove within which small narrow scales develop, similar to those around the female receptacle. Mucilage cells arise from the base of the antheridial chamber (figs. 16, 19). Usually the receptacle is raised only a slight distance above the general surface of the thallus (fig. 13), but it may, in some cases, be elevated on a short stalk (figs. 14, 15), the stalk being formed by intercalary growth of the ventral tissue forming a central core surrounded by air-chamber tissue like that of the thallus itself. The fungal hyphae of the compact tissue may penetrate the stalk, and in one case the raised portion bore a few ventral scales and a few pegged rhizoids on the posterior margin (fig. 14).



Figs. 13–15. Vertical longitudinal sections through the receptacles. Figure 13 shows two male receptacles (♂) and the female receptacle (♀), the male receptacles being sessile, as usual. Figures 14 and 15, male receptacle raised on a short stalk. Figure 14 shows the fungus-infected area (indicated by dotted lines) extending into the stalk, as well as small ventral scales (*sc*) and pegged rhizoids (*rh*) on the posterior margin. × 28.

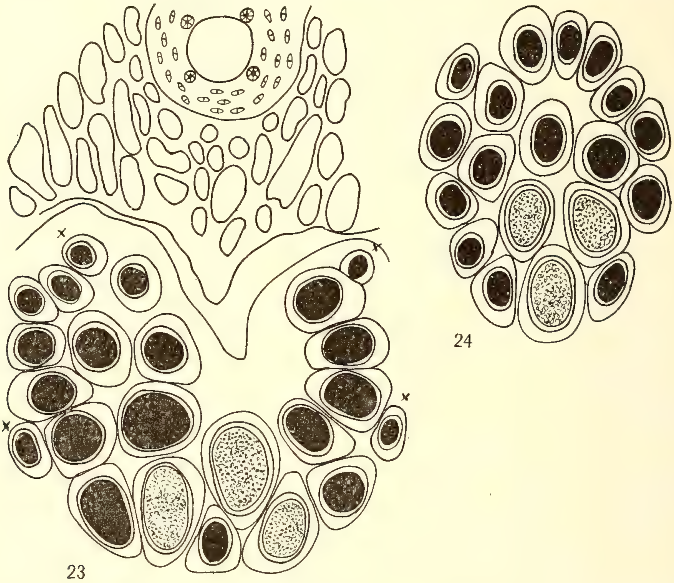
Bisexual Receptacles. Bisexual receptacles are not commonly found in the higher Marchantiales. They have been known for a long time in Chomiocarpon (Preissia), where they occur rather frequently, as the writer has found. They were first reported in *Dumortiera irrigua* by Taylor (27), and later for two other species, *D. trichocephala* and *D. velutina*, by Ernst (12). *Monoselenium tenerum*, as reported by Goebel (14), and occasionally certain species of Marchantia, according to Cutting (9), complete the list to date. The writer has found a few such cases in Reboulia, where archegonia may occur on the male receptacle (figs. 17, 18) or antheridia on a female receptacle (figs. 20–22). In the former case some of the antheridia were only partly enclosed by the chamber (fig. 19) or were raised above the surface of the disc by the elongated stalk (fig. 18). The young antheridia found on the female receptacle were deeply imbedded within the tissue. It is probable that bisexual receptacles will be found in still other forms of the Marchantiaceae, their significance lying in the occasional reversion to a primitive feature, and, as Cavers (8) suggests, they "demonstrate the ho-



FIGS. 16–22. Figure 16, median longitudinal section of a half-grown antheridium within the antheridial chamber. × 205. Figures 17 and 18, "male receptacles" with archegonia. × 82. Figure 19, young antheridium of figure 17. × 370. Figure 20, "female receptacle" with young antheridium (shown in black). × 82. Figures 21 and 22, young antheridia found in receptacle shown in figure 20. Figure 22 is the detail of the antheridium shown in figure 20. The section was cut obliquely across the antheridium. × 650.

mology which exists between the male and female receptacles throughout the Marchantiaceae."

Sexual Nature of the Plant. Most of the early writers of continental Europe speak of Reboulia as monoecious, occasionally dioecious. On the British Isles, according to both Lett (23) and Cavers (5, 6, 7), it is usually dioecious, occasionally monoecious, in the latter case with the two kinds of receptacles at the apices of different branches, agreeing with the observations of Leitgeb (21, 22). C. and R. Douin (10) claim Reboulia to be autoicous (monoecious), although it may appear dioecious by the abortion of either of the receptacles, but never paroicous. Howe (19) and Haupt (16) both find the American forms to be monoecious. The writer's observations show that the thallus may be sterile, male, female, or bisexual, with both sex organs on different branches or on the same branch, and even in the same receptacle, a variable condition indicating plasticity in this feature.



Figs. 23, 24. Transverse sections of male receptacles. Antheridia with mature sperms dotted, immature antheridia black. In figure 23, "*x*" indicates position of the youngest antheridia; female receptacle, with four archegonia, in the apical notch. Air chambers are shown anterior to the male receptacle. × 48.

The Antheridium

Origin. Haupt (16) says that "in all cases the antheridia develop strictly in acropetal succession from segments of the apical cell." The writer finds frequent exceptions to this rule. The first antheridia appear a few segments back of the apical cell and in the development of the receptacle are usually found near the median posterior margin (figs. 23, 24). An examination of the receptacle in transverse and vertical sections shows, however, a tendency toward a centrifugal origin of the sex organs. Young antheridia are found not only lateral, but even posterior to the oldest ones (figs. 14, 15, 23, 24). While, as shown in figure 23, there may be two general strands of sex organs, giving a lunate group, the youngest antheridia occur at the four points marked "*x*," indicating several possible points of origin of the sex organs. The oval disc (fig. 24) is not so definite in points of origin, yet shows a centrifugal development. That this is not merely due to different rates of development is shown by sections of young receptacles (Pl. XIV, figs. 26–29). Figures 26–28 show a young disc in whose development both anterior and posterior apical cells seem to be active, the oldest antheridia being near the center of the disc and the younger ones near the anterior and posterior margins. Oblique sections (fig. 29) and vertical transverse sections of the thallus, through the young disc, show the same condition. While the first antheridia may arise in close connection with the growing point of the thallus, the later ones, especially in a lunate group, may be far removed from the apex of the thallus, arising from apical cells along the margin of the disc (figs. 30, 31).

Development. The development of the antheridium, as a general rule, is essentially as given by Haupt (16), the initial arising as a papillate cell (Pl. XIV, fig. 25) which cuts off a basal cell (fig. 28). Other transverse divisions (fig. 29) result in a short filament, usually of four cells (fig. 32)—the limit, according to Haupt, but there may be as many as six (fig. 33), agreeing with the situation in Fimbriaria as described by Campbell (3). Vertical walls usually appear first near the center of the filament (fig. 34), and divide the segments into quadrants (fig. 41). Periclinal walls (figs. 35, 38–40) differentiate the wall from the primary spermatogenous cells. Transverse and vertical divisions of the basal portion build up the stalk (figs. 35, 38–40) which may become comparatively long (fig. 19) and is usually somewhat coiled within the chamber (fig. 16).

In some cases the vertical wall of the terminal cell is strongly inclined (fig. 36). The next wall, cutting at an angle to this, produces an apical cell with two cutting faces from which a few segments may be cut off (figs. 38–40). The writer has also observed this occasionally in Chomiocarpon and Marchantia. This feature suggests the antheridium of Sphagnum and of the Bryales. Inner cells from these segments make their contribution to the spermatogenous tissue (figs. 39, 40, 16). Cross sections of the young antheridia show that in addition to the usual method of differentiation of the wall and primary spermatogenous tissue (figs. 41, 42), interesting variations may occur (figs. 43, 44), in some cases resulting in only two primary spermatogenous cells in the segment and resembling the condition found in the Jungermanniales (fig. 44). Hutchinson (20) showed a considerable variation in the development of the antheridium of *Pellia epiphylla*, both the so-called Marchantia and Jungermannia types occurring. These variations indicate that Reboulia is a plastic form in the development of the antheridium.

The divisions of the primary spermatogenous cells follow no regular plan (figs. 40, 45, 46), although their original boundaries can be detected for a considerable time in the development of the tissue (fig. 16), especially when division takes place, as all the offspring of a primary spermatogenous cell usually divide simultaneously. The wall is a single layer of cells and retains its form until the maturity of the sperms. The young antheridia are somewhat erect, but by the time of maturity have become strongly inclined (figs. 13, 16).

The Morphological Nature of the Receptacle

There have been two interpretations of the male receptacle of Reboulia. The one, first suggested by Hofmeister (18), regards it as a shoot; the other, put forth by Leitgeb (21, 22), holds it to be merely a dorsal outgrowth. This latter view is based on the claim that the apex of the thallus is not used up in its formation. Even if the thallus is permanently checked, the apex is thought to be evident below the disc. Leitgeb regards the lunate disc as merely a repetition of the form of the thallus notch. Cavers (6, 7), supporting the former idea, says:

> The male receptacle of Reboulia may be regarded as representing a branch, having on its anterior margin a single growing point, or, in many cases, two growing points. Apart from the fact that the branching in the latter case takes place at a late stage, after several antheridia have been formed, and occurs once only, giving rise to a crescent-shaped receptacle, there is no essential difference between the receptacle of Reboulia and that of Fegatella, in which the branching takes place at an earlier stage and is repeated several times, giving rise to radiating rows of antheridia.

He holds the growing point to be used up in the formation of the receptacle, and in some cases to branch, giving the two horns of the disc. The continuation of the thallus in front of the receptacle is regarded as an innovation shoot. Goebel (14) from his study of *Monoselenium tenerum*, a monoecious form with the male receptacle behind the female, which latter may also become dorsally placed, finds that the sessile male disc may be elevated on a short stalk with scales and rhizoids, but without a rhizoid groove. Both receptacles in Monoselenium, according to Goebel, represent branch systems, the antheridia developing in centrifugal order on the upper surface. He regards the forward growth of the thallus as an adventitious branch. From Goebel's claim that the receptacle of a "branch-system" type may become dorsal Cavers (8) concludes that a

> Composite receptacle . . . need not necessarily terminate the growth of the thallus but may come to occupy the same position as the "dorsal outgrowth" type of receptacle which Leitgeb regarded as contrasting so strongly with the "branch-system" type.

Goebel (14, 15) would explain the dorsal sessile receptacles, such as occur in Reboulia and Plagiochasma, as reduced from those of forms such as

Marchantia and Chomiocarpon, the dorsal position being due to the very early appearance of the adventitious branch. The male and female receptacles, according to this, are morphologically equivalent, Goebel holding that receptacles of different morphological nature are improbable on the same plant. Voigt (28), Leitgeb (21, 22), and Cavers (7) all found female receptacles in Reboulia in a median dorsal position behind the apex, conditions giving support to the homology of the two receptacles, despite the abnormality of such a position of the female receptacles. Evans (13), discussing the homology of the female receptacle in Plagiochasma and Reboulia, says:

> The mere fact that the growing point is not carried upward by the elongating stalk (in Plagiochasma) does not invalidate the homology of the carpocephalum with that of Reboulia.

His conclusion could be applied to the male receptacle as well. Haupt (16) finds the activity of the apical cell unchecked in the formation of the disc and apparently holds to Leitgeb's view.

It was shown above that the disc may be elevated on a short stalk which may, in rare cases, bear scales and pegged rhizoids. The presence of one or more growing points on the young receptacle is evident, as well as is the formation of apical cells on the disc independent of the apex of the thallus (fig. 30). The writer is inclined to the view that in the male receptacle of Reboulia we have a possible elementary stage of a branch system, representing a transition from the "dorsal outgrowth" to the "composite" form, or *vice versa* if one were to insist on a reduction series. Reboulia is sufficiently plastic to give several phases of the transition, as, for example: the thallus apex may be unchecked, the sessile male receptacles frequently dorsal, the female receptacle occasionally dorsal; the growth of the thallus may be checked temporarily by the formation of the male receptacle, an innovation shoot growing out with a narrow base, giving the jointed aspect of the plant sometimes figured, as by Goebel (15); the thallus apex may be permanently checked, being used up in the formation of the male receptacle, which remains as a sessile structure (as in Lunularia and Conocephalum); and, rarely, the male receptacle may be elevated on a short stalk (figs. 14, 15). The marginal male receptacles, as found by C. and R. Douin (10), and the occasional clusters (figs. 3, 4) can be explained as due to intercalary growth following the forking of the disc, thus separating the several points of origin from one another.

Conclusion

On the whole it seems to the writer that the male receptacle and the antheridium of Reboulia suggest a very plastic and significant condition from a morphological standpoint, being primitive in some features and suggestive of higher forms in others.

SUMMARY

1. The male receptacle of *Reboulia hemisphaerica* occupies a dorsal position, as a rule, posterior to the female receptacle which terminates the branch.

2. The receptacle is usually more or less lunate in outline, although it may be circular, or more or less irregular. It is sessile, as a rule, although in rare cases it may be elevated on a very short stalk.

3. *R. hemisphaerica* is monoecious, with the sex organs in distinct groups, although bisexual receptacles may occur.

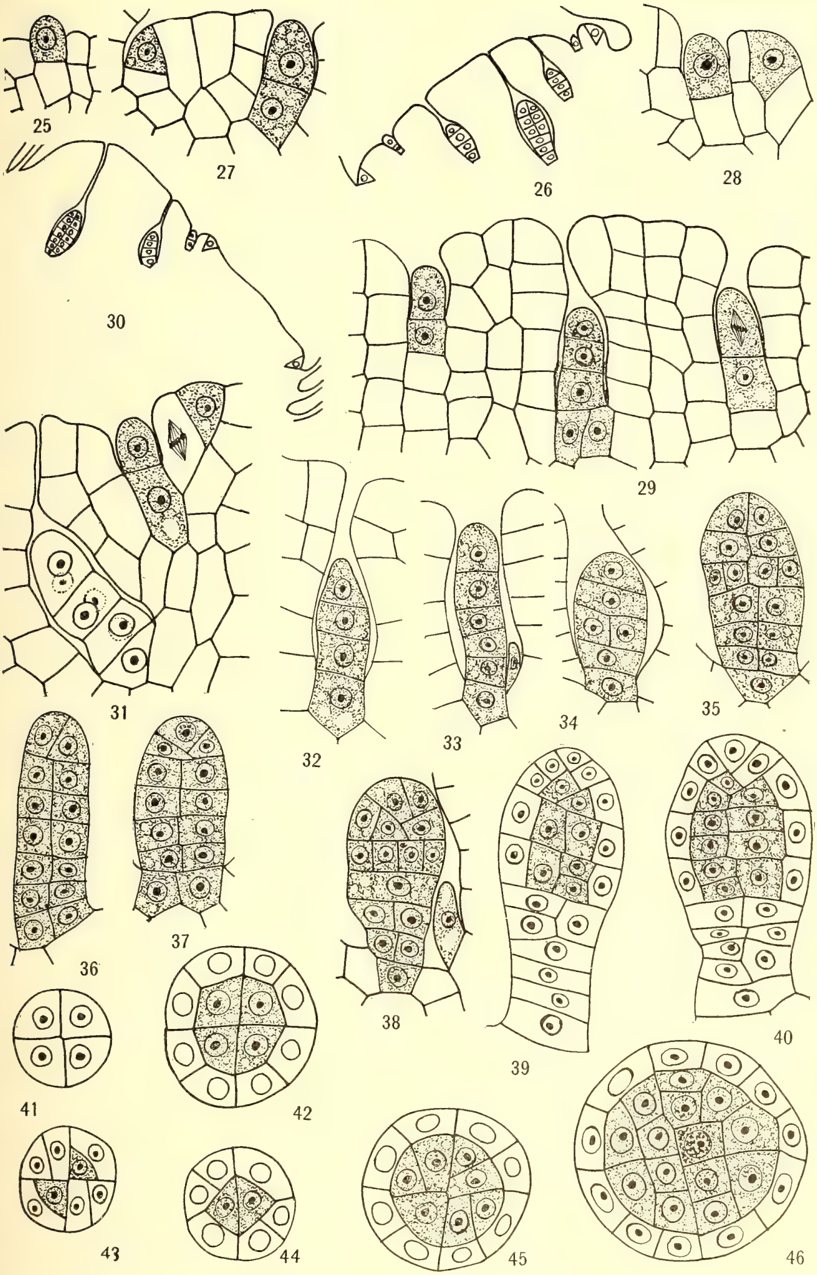
4. The antheridia show a tendency toward centrifugal development. Variations from the usual marchantiaceous type of development occur, such as the occasional appearance of an apical cell with two cutting faces and the occasional formation of only two primary spermatogenous cells in a segment.

5. The male receptacle is a plastic structure, probably representing an elementary stage of a branch system and showing transitions from the "dorsal outgrowth" type to the "composite branch-system" type.

JUNIATA COLLEGE,
HUNTINGDON, PENNSYLVANIA

LITERATURE CITED

1. **Bischoff, G. W.** Bemerkungen über die Lebermoose, vorzüglich aus den Gruppen der Marchantieen und Riccien. Acta Acad. Caes. Leop. **17**^{2} : 908–1088, pls. 67–71. 1835.
2. **Boulay, N.** Muscinées de la France II. Hépatiques. (Cited by Douin.)
3. **Campbell, D. H.** Mosses and ferns. 3d ed. New York, 1918.
4. **Cavers, F.** Explosive discharge of antherozoids in Hepaticae. Torreya **3** : 179–182. 1903.
5. ——. On the structure and biology of *Fegatella conica.* Annals of Bot. **18** : 87–120, figs. 28–32, pls. 6, 7. 1904.
6. ——. Contributions to the biology of the Hepaticae. Part I. Targionia, Reboulia, Preissia, Monoclea, pp. 47, figs. 1–12. Leeds and London, 1904.
7. ——. Notes on Yorkshire Bryophytes III. *Reboulia hemisphaerica* (L.) Raddi. Reprint from The Naturalist, pp. 15, pl. 8, figs. 2–6. 1904.
8. ——. Interrelationships of the Bryophytes. New Phytol. reprint no. 4, pp. 203, figs. 1–72. 1911.
9. **Cutting, E. M.** On androgynous receptacles in Marchantia. Annals of Bot. **24** : 349–357. 1910.
10. **Douin, C., and Douin, R.** Le Reboulia Raddi. Rev. Gén. Bot. **30** : 129–145, figs. 1–5. 1918.
11. **Dupler, A. W.** The air chambers of *Reboulia hemisphaerica.* Bull. Torrey Bot. Club **48**: 241–252, figs. 1–22. 1921.
12. **Ernst, A.** Über androgyne Inflorescenzen bei Dumortiera. Ber. Deutsch. Bot. Ges. **25** : 455–464, pl. 13. 1906.
13. **Evans, A. W.** The genus Plagiochasma and its North American species. Bull. Torrey Bot. Club **42** : 250–308, figs. 1–8. 1915.
14. **Goebel, K.** Archegoniatenstudien XIII. *Monoselenium tenerum.* Flora **101** : 43–97, figs. 1–45. 1910.
15. ——. Organographie der Pflanzen II. Spezielle Organographie 1. Bryophyten. Jena, 1915.


25
27
26
28
30
29
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

DUPLER: REBOULIA HEMISPHAERICA

16. **Haupt, A. W.** Gametophyte and sex organs of *Reboulia hemisphaerica.* Bot. Gaz. **71** : 61–74, figs. 1–21. 1921.

17. ——. Embryogeny and sporogenesis in *Reboulia hemisphaerica.* Bot. Gaz. **71** : 446–453, pl. 33, figs. 1–11.

18. **Hofmeister, W.** On the higher Cryptogamia. Eng. ed. London, 1862.

19. **Howe, M. A.** Hepaticae and Anthocerotes of California. Mem. Torrey Bot. Club **7.** 1899.

20. **Hutchinson, A. H.** Gametophyte of *Pellia epiphylla.* Bot. Gaz. **60** : 134–143, fig. 1, pls. 1–4. 1915.

21. **Leitgeb, H.** Die Infloreszenzen der Marchantiaceen. Sitzsber. Kais. Akad. Wiss. **81** : 123–143. 1880.

22. ——. Untersuchungen über die Lebermoose VI. Die Marchantieen. Graz, 1881.

23. **Lett, H. W.** Hepatics of the British Islands. 1902.

24. **Micheli, P. A.** Nova plantarum genera. Florence, 1729.

25. **Nees ab Esenbeck, C. G.** Naturgeschichte der europäischen Lebermoose 4. Breslau, 1838.

26. **Stephani, F.** Species Hepaticarum. Bull. Herb. Boissier **6** : 790–799. 1898.

27. **Taylor, T.** De Marchanteis. Trans. Linn. Soc. **17** : 375–395, pls. 12–15. 1836.

28. **Voigt, A.** Ein Beitrag zur vergleichenden Anatomie der Marchantiaceen. Bot. Zeit. **37** : 729–743, 745–759, pl. 9. 1879.

29. **Woodburn, W. L.** Preliminary notes on the embryology of *Reboulia hemisphaerica.* Bull. Torrey Bot. Club **46** : 461–464, pl. 19. 1919.

EXPLANATION OF PLATE XIV

All drawings were made with a camera lucida and have been reduced one half in reproduction.

FIG. 25. Antheridium initial. × 650.

FIG. 26. Section of a young male receptacle, showing the centrifugal development of antheridia. × 155.

FIGS. 27, 28. Detail of the anterior and posterior margins, respectively, of figure 26; figure 28 shows antheridium initial; figure 27, basal cell cut off. × 650.

FIG. 29. Portion of a receptacle showing younger antheridia on either side of the older one. × 650.

FIG. 30. Section of a young receptacle with an apical cell at the anterior margin of the disc, some distance posterior to the apical cell of the thallus. × 155.

FIG. 31. Detail of a portion of figure 30. × 650.

FIGS. 32–35. Development of young antheridia. × 650.

FIGS. 36–40. Young antheridia with apical cell of two cutting faces. × 650.

FIGS. 41–46. Transverse sections of young antheridia. × 650.

THE MEIOTIC CYTOKINESIS OF NELUMBO

Clifford H. Farr

(Received for publication November 3, 1921)

The division of the cell is a phase of cytology which has proved to have a wide application to other branches of biological science. The division of the nucleus through the phenomena of karyokinesis and other chromatin behaviors has been found to be of direct importance to the subject of genetics. The partitioning of the cell, or cytokinesis, has on the other hand thrown light on the dynamics of the cell and cell physiology and is also closely related to growth, a very important phase of physiological investigation. The recognition of the fact that growth embraces not only cell enlargement, but cell division and cell differentiation, and also, in the multicellular organism, intercellular stresses and strains, warrants a renewed attack upon the field of cytokinesis. I. W. Bailey (1–5) has recently been making an extensive study of cell-plate formation in the cambium, and has shown very clearly how cell-plate formation may be adapted to the longitudinal division. of very much elongated cells. Another line of investigation of cytokinesis in plants has been the establishment of the existence of cell division without cell plates but by a furrowing process in the formation of the microspores of certain Angiosperms. This was demonstrated by the writer first in Nicotiana, Primula, Helianthus, Ambrosia, Tropaeolum, and Chrysanthemum (6), and later in Magnolia (7) and Sisyrinchium, a monocotyledon (8). Mrs. W. K. Farr has also found the same procedure in Cobaea (9).

While the writer's first paper (6) was in press, Tahara (20) published a paper entitled "Cytological Studies on Chrysanthemum" in which he states that

> At the end of the meiotic nuclear division, the new partition cell walls appear in the form of protuberances in the inner surface of the cell wall of the pollen mother cells. These protuberances proceed centripetally and constrict the pollen mother cell into four equal portions. This type of tetrad division reminds us of the type of tetrad division of the tetraspores in Rhodophyceae.

No further description or discussion is given, and no figures of these stages appear in that paper. He does not state that a cell plate is absent, nor does he discuss the relation of the plasma membrane to the process. His statements given above, however, make it clear that he considers it a genuine furrowing and not simply a rounding up of the cells after division. Very recently Tahara (21) has published again on this subject, this time including six text figures of quadripartition in Chrysanthemum. These figures resemble very closely the figures which I published of Nicotiana (6), but he does not refer to any of my papers. He distinguishes three types of tetrad formation, namely: the dicotyledonous type, the monocotyledonous type, and the rhodophyceous type. The last-named is obviously the quadripartition by furrowing, the monocotyledonous type is that of successive bipartition by cell plates, and the dicotyledonous type is apparently held to be quadripartition by cell plates, though he gives no forms in which such a process occurs nor does he refer to any papers presenting this type of division.

Gates and Rees (10) have just published their complete study of Lactuca in which they find quadripartition by furrowing, which they describe as exactly like that which I found in Nicotiana. They give five excellent figures of the stages of cytokinesis of the pollen mother cells.

Several Swedish investigators have been extending very rapidly our knowledge of the occurrence of quadripartition and successive bipartition respectively in the division of the pollen mother cells of monocotyledons. To the work of Täckholm and Söderberg (18, 19) have been added now two papers, one by Söderberg (17) and another by Palm (15) just last year. Söderberg (17) presents a list of more than 73 species of monocotyledons studied by himself and others. More than 38 have quadripartition, and more than 34 have successive bipartition. Quadripartition, he reports, is found in seven families of monocotyledons and successive partition in four. He himself in this paper reports the first case of quadripartition in a palm, namely *Chamaedorea corallina*. Palm (15) adds to the list of Söderberg observations on 8 additional families and on 19 species of families which have already been studied to some extent. It thus appears that up to the present successive bipartition and quadripartition have both been found in four families of monocotyledons; quadripartition alone has been found in six families; and bipartition alone has been reported in eighteen families. In the first group are the Naiadaceae, Liliaceae, Commelinaceae, and Orchidaceae. In the second group are the Juncaceae, Dioscoreaceae, Iridaceae, Taccaceae, Cyperaceae, and Palmae. Although Palm (15) does not take up a careful cytological study of the details of the process, he mentions a few observations which are in harmony with my findings. In the two species of Stemona in which he found successive bipartition he mentions that the mother cell walls are very thin. In Dianella, a lily, which has quadripartition, he reports that "no traces of a cell plate could be seen during the heterotypic division"; whereas in certain of the Amaryllidaceae having successive bipartition he reports that a conspicuous cell plate is usually present in the first division.

In 1907 Lubimenko and Maige (13) described the cytokinesis of the pollen mother cells of the two water lilies, *Nymphaea alba* and *Nuphar luteum*. In the former they found and figured the complete formation of a cell plate after the first division of the nucleus, though they state that it may never entirely extend to the plasma membrane on all sides. This cell plate disappears during interkinesis. In *Nuphar luteum* no cell plate at all is developed after the heterotypic karyokinesis, though they report in extremely rare instances that a hyaline line may be seen across the equator. In *Nymphaea alba* they find a transitory cell plate appearing in the late anaphase of the homoeotypic mitosis, and their figure 51 substantiates this observation. No such transitory plate is found in *Nuphar luteum*. Their figures 53 and 52 show the tetranucleate stage of these two species respectively, with no partitions or cell plates present. Their figure 54 is of a pollen mother cell of *Nymphaea alba* after the partitions are completely formed separating the four cells. No further figures are given of cytokinesis. They describe the formation of the partitions as follows:

> Se forment brusquement les plaques cellulaires. Ce phénomène se produit très rapidement et on n'aperçoit, sur les coupes, que les plaques entièrement formées.

They interpret the disappearance of the transitory cell plate as due to the fact that the spindle fibers upon which it is formed are of nuclear origin, and that the material of which they are composed is used again in the formation of the linin thread and nucleoli of the new nuclei. The cell plate, which they believe is formed later and finally accomplishes division, is considered as being formed on spindle fibers of cytoplasmic origin appearing after the disappearance of the transitory cell plate.

Several considerations make it seem advisable to investigate further the cytokinesis of the pollen mother cells of some of the Nymphaeaceae. According to the work of Lubimenko and Maige (13), it would seem that *Nymphaea alba* resembles Magnolia in the existence of a transitory cell plate after the heterotypic nuclear division, but that it differs from Magnolia in that no incipient furrow is developed at this stage. *Nuphar luteum*, on the other hand, seems to correspond exactly to other flowering plants having quadripartition in so far as the events immediately following the first division of the nucleus are concerned. Furthermore, the instance of the disappearance of a transitory cell plate and the later formation of a permanent cell plate on the same spindle reported by these authors for Nymphaea apparently stands alone in the literature as the only case of such a behavior, and in itself would warrant further investigation.

For these reasons I collected pollen mother cells of *Nelumbo lutea* (Willd.) Pers. on July 9, 1921, at the Amana colonies in Iowa County, Iowa, where they grow luxuriantly in a large pond (text fig. 1). On this date large numbers of the flowers were in full bloom, but the blooming season extended on into August so that it is likely that the flowers used in this study would have bloomed in about mid-season. Reduction divisions occur in the pollen mother cells of this species when the buds are about one inch in diameter and about one and one half inches long. At this time the bud is between one and two feet above the water, the peduncle elongating only a few inches more before its growth ceases. In this, as well as in the forms which I have previously studied, it has been found that the stages of the reduction divisions immediately precede the appearance of pigment in the anther, the yellow or orange tinge showing just after the microspores are formed. This fact aids much in readily finding the particular stages which are desired. After the examination of the pollen mother cells of one or more stamens from a flower in living condition under a compound microscope in the field, other stamens from the same flower were fixed in Flemming's solution. In this way the desired stages were secured, and a large amount of imbedding, cutting, and staining in order to find the right stages was eliminated. The tip of the stamen projecting beyond the anthers, which is characteristic of this genus, was cut away before fixation in order to permit of ready penetration of the fixing solution into both ends of the stamens.

TEXT FIG. 1. The bed of *Nelumbo lutea* from which the material for this study was collected.

A longitudinal section of the stamen of *Nelumbo lutea* (Pl. XV, fig. 1) shows that the anthers are very long and slender. In most cases there is space for not more than four or five pollen mother cells to lie side by side across the pollen chamber in its widest place. During the reduction divisions the mother cells are usually not in contact with each other, but in a region where there is room for four cells to lie side by side across the anther not more than three will be found. During presynapsis and the early stages of synapsis this large amount of intercellular space is filled with a colloidal matrix which disappears near the middle of the synaptic period and leaves the mother cells free within the anther during the remainder of the reduction divisions. Just after the disappearance of the colloidal matrix the mother-cell walls begin to thicken. Whether or not the material of the colloidal matrix contributes to the thickening of the wall, or whether the water released by dissociation of the intercellular colloid is subsequently adsorbed by the gelatinized cell wall of the mother cells, has not been determined. It may be that there is no relation between the disappearance of the gel between the cells and the thickening of the cell wall.

A peculiarity of the thickening of the walls of the mother cells of *Nelumbo lutea* is the high degree of inequality in the thickening on the various sides of the same cell. It is usually found that the sides of the cells toward the ends of the anthers are much more thickened than those toward the lateral surfaces. By the time the stage of interkinesis between the heterotypic and homoeotypic divisions is reached, it is found in some cases that the thickening of the wall on each of the two ends is equal to at least one half of the diameter of the cell lumen, whereas the walls on the lateral sides are scarcely thickened at all. Another feature noted about the anther of this species is that the tapetum usually remains living and intact throughout the reduction process. It consists commonly of very large cells, in some cases almost as large as the pollen mother cells. They may contain more than one nucleus, and mitotic figures are of common occurrence within them.

In Magnolia, as previously reported (7), the different stages of the reduction divisions may be found in the same anther, and there seemed to be no special arrangement or order of progression from one part of the anther to others. In Sisyrinchium (8), a very definite graded series exists from one end of the anther to the other. Either the distal or the proximal end of the anther may be more advanced, but there is a regular succession of stages, the whole range of stages in any anther not being very great. In *Nelumbo lutea* an intermediate condition exists. There is a succession of stages from one part of the anther to another, but it is not always from one end of the anther to the other. Furthermore, the range of stages found within a given anther is frequently much larger than in Sisyrinchium. Cases were found in which the tetranucleate condition was found at one end and the microspore stage at the other, with a series showing quadripartition in successive degrees of advancement between. In other cases diakinesis was found at one end of the pollen chamber and the tetranucleate condition at the other. In some cases both ends of the anther are more advanced than the middle. The middle portion may show interkinesis while the ends are in the tetranucleate stage; or the reverse may be true, with tetranucleate cells in the middle and interkinesis or even diakinesis at both ends. It might be well at this place to explain the term "interkinesis," which I employed in my recent paper on Sisyrinchium (8) and am using again in the present paper. In 1912 Lundegårdh (14) introduced the term "interphase" to refer to the interval between two successive mitoses. He used it, however, in all cases to refer to the condition of the nucleus during that interval, making it coördinate with "prophase," "metaphase," etc. Sharp (16) in 1914 called attention to this meaning of the term, so that it now seems well established in cytological literature. It now appears that we need a term to refer to the condition of the entire cell between the time of the completion of cytokinesis and the initiation of the next succeeding karyokinesis. It is evident that the interphase condition of the nucleus may begin during cytokinesis, so that a new term coördinate with karyokinesis and cytokinesis is required, and "interkinesis" seems to be the logical choice. Throughout the study of *Nelumbo lutea* it was found that the karyokinetic stages in the reduction divisions seemed to be relatively few, whereas the cytokinetic and interkinetic stages were quite prevalent. This leads to the conclusion that karyokinesis proceeds in this form much more rapidly than cytokinesis, which hardly agrees with the findings of Lubimenko and Maige (13) in other species of this family.

The metaphase of the heterotypic division presents a very long spindle with a very narrow equatorial plate of chromosomes. The spindle is usually quite straight, though cases were noted where it curves gently at the poles. As nearly as could be determined the number of chromosomes is eight, though in some cases apparently good polar views revealed not more than five or six. This would of course be the gametophytic number. Apparently the number of chromosomes has never before been counted in this species. In 1898 Guignard reported 32 chromosomes in *Nymphaea alba*, and the following year Strasburger reported 48 for the same species. In 1897 Guignard reported 16 as the diploid number in *Nuphar luteum*, while Lubimenko and Maige in 1907 and Rosenberg in 1909 agree that the haploid number is 17.

The halves of the dyad chromosomes pull apart in the anaphases and pass to the poles in the usual manner. In the telophases the distance between the two plates of chromosomes is usually equal to or greater than the distance from either of these to the plasma membrane at its nearest point. Very soon after the chromosomes take this position the spindle fibers become apparently thicker along their middle portions. Whether this is due to a real thickening of the fibers themselves, or whether it is more or less of an illusion brought about by the crossing of fibers which lie nearly parallel to each other, is difficult to determine. But it soon becomes evident that a real thickening of the individual fibers has occurred, as there is formed a cell plate of these thickenings. These thickenings, which at first appear spaced, thicken until they touch each other, making a continuous layer. It does not extend beyond the limits of the central spindle, however, and hence is to be considered as an incomplete cell plate. This incomplete cell plate may persist after the nuclei are completely reorganized, but it always disappears during early interkinesis at least. In no instance was a pollen mother cell in interkinesis or subsequent stages found in which complete bipartition had taken place.

Interkinesis is marked by the disappearance of the transitory cell plate and the subsequent enlargement of the two nuclei. The spindle fibers become progressively fewer and fewer, until in some cases it is practically impossible to find a single fiber connecting the two nuclei. The fibers are furthermore obscured by the formation of numerous large bodies in the cytoplasm during this period. These bodies approach the size of plastids, though they do not seem to have as definite boundaries as do those structures. They seem to be slightly flocculent in consistency, and it may be that further studies in chrondriosomes will reveal their nature.

The incomplete cell plate of Nelumbo seems to resemble that of Magnolia (7) to a considerable extent, except that it is perhaps somewhat more fully formed. Timberlake (23) reported such a structure as occasionally being found in the larch. He explains the failure to accomplish partition in these cases as due to a lack of formation of peripheral spindle fibers, and the same might be said of the condition in Nelumbo. Tangl (22) and Juel (11) found incomplete cell plates in Hemerocallis, but here they persist for some time after the telophases. A closer approach to the condition in Nelumbo is probably that reported by Juel (12) in Carex as occurring after both the heterotypic and the homoeotypic divisions.

The period of interkinesis is apparently quite long, and during this time the nuclei enlarge very much. They become very much larger than the nuclei which result from the second division, and can be easily distinguished from the latter by their size without consulting neighboring sections to determine the number of nuclei within the cell. These nuclei in interkinesis remain at some distance from the plasma membrane. No evidence at all of an incipient furrow such as was found in Magnolia (7) was revealed.

The homoeotypic mitosis is accomplished by the formation of long, narrow spindles which appear very much like those of the heterotypic mitosis except that they are smaller. In some cases the two spindles are almost exactly parallel, while in others they are almost exactly at right angles (Pl. XV, fig. 2). It seems likely that the usual condition is that the spindles are somewhere between parallel and at right angles to each other. At any rate, the division results usually in a tetrahedral arrangement of microspores, though there are some departures from this disposition.

Observations were made on many cells showing the homoeotypic telophase spindles at various stages, but in no case was a cell plate or orange zone seen. When the chromosomes first reached the poles, there was in one or two cases an apparent thickening of the spindle fibers throughout the middle portion of their length, just as was observed in the heterotypic division. But in each case I was able to satisfy myself that this was simply due to the crossing of the fibers in this region. It is evident that if the halves of two homologous chromosomes lie side by side at the poles as they do in telophase, and if the halves of each pair are connected by spindle fibers, these fibers will cross and may even touch in the equatorial plane. In this case the appearance will be that of a much attenuated letter *X* with the upper and lower angles of the figure very small and the lateral angles very large. This is exactly the appearance which the equatorial plane of the central spindle in some of these cells presents. It is not followed by the obvious thickening of the fibers such as one finds in the heterotypic division, and consequently could not be taken as evidence of the development of a cell plate. Figure 2 is of a cell which is precisely at the stage when the early stages of cell-plate formation should be taking place, if they are to occur at all. It is at this stage of the first division that the transitory cell plate puts in its appearance, and in all other forms that I have studied the cell plate if it is formed at all is associated with this stage of the karyokinesis.

As the nuclei become organized they do not take the form of flat discs such as are found in Magnolia (7), but round up directly (fig. 3). As they round up and enlarge, the spindle fibers become fewer and fewer. No cases were found in which spindle fibers appear to be entirely lacking as in the stages of interkinesis, but they do become very scarce indeed. It is obvious that if a cell plate were being formed there would in all probability be a great increase in the number of spindle fibers especially in the peripheral region, but such is not found to be the case. The nuclei gradually migrate toward the plasma membrane as they enlarge, in a very similar manner to that found in Nicotiana (6). It must be that a relatively long period of time is involved in this stage of the formation of pollen. This is indicated by the frequency of these stages in the sections studied, and also by the enormous enlargement of the nuclei and their migration to the plasma membrane. An examination of figures 2, 3, and 4 suggests the degree of this enlargement. The volume of the nuclei shown in figure 4 is approximately four times that of the nuclei shown in figure 3. Furthermore, the cell itself has undergone an enlargement, that shown in figure 4 being about twice as large as that shown in figure 3. Even more marked than either of these changes is the enlargement of the nucleoli, which in the latter stage are many times their former size. The prochromosomes so distinct in the earlier stage are now quite indefinite in appearance. All the evidence seems to indicate that a long interval elapses between the completion of karyokinesis and the initiation of cytokinesis.

Following this, which is called the tetranucleate stage, there occurs the quadripartition of the cell by furrowing. This begins by the appearance of a structure which may at first seem to be a centripetally forming cell plate. A chip from the superficial portion of the protoplast may make it appear that there is a continuous plate across the equatorial plane. Such a view is shown in the upper part of the cell of figure 4, where two spindles are crossed by continuous plates. That this is not a central section of the spindle but a superficial view is obvious from the fact that the upper nucleus does not appear in this section. But it is equally apparent that the appearance of the equator of the two upper spindles would be exactly the same in their central portion if the section had been cut so as to show a chip off the upper nucleus and similar chips off the two lower nuclei. Such a section would give evidence of a continuous plate across the central spindle, until a careful examination of adjacent sections showed this interpretation to be incorrect. It is doubtless this mistake which was made by Lubimenko and Maige and by other observers in reporting at least some of the instances of quadripartition by cell plates. A central section and careful focusing invariably, in all instances which I have seen, reveal the fact that this plate does not extend across the center of the spindle and that it is connected with the plasma membrane at its periphery.

From the time the furrow begins on the very boundary of the cell, there is an increase in the number of fibers of the central spindle. They seem to appear in centripetal order rather than centrifugally as in typical cell-plate formation. The furrow is very slender indeed, which adds to its resemblance to a cell plate as compared with the furrows of the other plants which I have studied. It is so thin, in fact, that it appears to consist during its development simply of the plasma membrane itself, and that cell-wall material is not found between the daughter cells until later. However, observation of cells which are somewhat plasmolyzed indicates that this interpretation is incorrect, and that a projection of the cell wall does extend into the furrow in some cases at least. Even at this stage of quadripartition the cell walls are usually not thickened to a marked degree on all sides. Figure 4 shows an enormous thickening of the wall above and a considerable thickening below, but the sides of the cell wall are rather thin. The middle lamella of the old mother cell is quite evident in some instances (fig. 4), while in other cases it seems to be lacking (figs. 2, 3).

A superficial view of the furrow indicates that it is perforated, as if it were made up of thickenings on spindle fibers as in typical cell-plate formation. Careful observation, however, reveals the fact that this perforated appearance obtains only on the inner margin of the centripetally forming furrow. Mrs. Farr (9) in her study of Cobaea found that the furrow as it advances inward presents not a simple cutting edge, but that its edge is wavy, the projections extending in between the fibers of the central spindle. Such a condition would give the appearance in section which is described above.

During quadripartition the furrows are apparently all simultaneously initiated within a given mother cell and proceed in their development at about the same rate, so that it is the portion of the central spindles between the exact central point and the exact center of the entire tetranucleate cell which is the last to be traversed by the furrow. There are six spindles and four furrows as in Nicotiana (6), and the furrowing usually proceeds a little faster at the region of the junction of the three furrows, so that the furrowing may extend to the geometrical center of the cell before it has completely traversed the spindles. This situation was found in a considerable number of cells observed.

The mother-cell wall becomes more uniformly thickened on all sides as the microscope stage approaches. As noted above, the microspores when first formed are separated by very thin plates consisting of the two plasma membranes and a very thin layer of wall material. The spindle fibers are still quite conspicuous at this time, as was also noted by Lubimenko and Maige in the forms they studied (13). As the spindle fibers disappear the microspores slowly round up, the wall material becoming more abundant at the periphery at first. It is not clear whether this consists in the swelling of the material already in place or in the intrusion of additional material from the older portions of the surrounding wall. Although the rounding-up process takes place more slowly than in any form which I have previously studied, yet it is finally carried forward to the same extent as in the other forms.

LITERATURE CITED

1. **Bailey, I. W.** Phenomena of cell division in the cambium of arborescent gymnosperms and their cytological significance. Proc. Nat. Acad. Sci. **5** : 283–284, fig. 1. 1919.
2. ——. The formation of the cell plate in the cambium of the higher plants. Proc. Nat. Acad. Sci. **6** : 197–200, 8 figs. 1920.
3. ——. The cambium and its derivative tissues II. Size variations of cambial initials in gymnosperms and angiosperms. Amer. Jour. Bot. **7** : 355–367, figs. 1, 2. 1920.
4. ——. The cambium and its derivative tissues III. A reconnaissance of cytological phenomena of the cambium. Amer. Jour. Bot. **7** : 417–434. Pls. 26–29. 1920.
5. ——. The significance of the cambium in the study of certain physiological problems. Jour. Gen. Physiol. **2** : 519–533. Pl. 1, text figs. 1–4. 1920.
6. **Farr, C. H.** Cytokinesis of the pollen mother cells of certain Dicotyledons. Mem. N. Y. Bot. Garden **6** : 253–317. Pls. 27–29. 1916.
7. ——. Cell division by furrowing in Magnolia. Amer. Jour. Bot. **5** : 379-395. Pls. 30–32. 1918.
8. ——. Quadripartition by furrowing in Sisyrinchium. Bull. Torrey Bot. Club **49** : 51–62. Pl. 2. 1922.
9. **Farr, Wanda K.** Cell division of the pollen mother cell of *Cobaea scandens alba*. Bull. Torrey Bot. Club **47** : 325–338. Pl. 14. 1920.
10. **Gates, R. R., and Rees, E. M.** A cytological study of pollen development in Lactuca. Annals of Bot. **35** : 365–398. Pls. 16–19. 1921.
11. **Juel, H. O.** Die Kerntheilung in den Pollenmutterzellen von *Hemerocallis fulva*. Jahrb. Wiss. Bot. **30** : 205–226. Pls. 6–8. 1897.
12. ——. Beiträge zur Kenntnis der Tetradenteilung III. Die Entwicklung der Pollenkörner bei Carex. Jahrb. Wiss. Bot. **35** : 649–656. Pl. 16, figs. 28–44. 1900.
13. **Lubimenko, W., and Maige, A.** Recherches cytologiques sur le développement des cellules-mères du pollen chez les Nymphéacées. Rev. Gén. Bot. **19** : 474–505. Pls. 1–5, text figs. 1, 2. 1907.

14. **Lundegårdh, H.** Das Caryotin im Ruhekern und sein Verhalten bei der Bildung und Auflösung der Chromosomen. Arch. Zellforsch. **9** : 205–330. Pls. 17–19. 1912.
15. **Palm, D.** Preliminary notes on pollen development in tropical monocotyledons. Svensk Bot. Tidskr. **14** : 261–266. 1920.
16. **Sharp, L. W.** Somatic chromosomes in Vicia. Cellule **29** : 295–328. Pls. 1, 2. 1914.
17. **Söderberg, E.** Über die Pollenentwicklung bei *Chamaedorea corallina* Karst. Svensk Bot. Tidskr. **13** : 204–211. 1919.
18. **Täckholm, G., and Söderberg, E.** Über die Pollenentwicklung bei Cinnamomum nebst Erörterungen über die phylogenetische Bedeutung des Pollentyps. Ark. Bot. **15**^{8} : 1–12. 1917.
19. ——. Neue Beispiele der simultanen und sukzessiven Wandbildung in den Pollenmutterzellen. Svensk Bot. Tidskr. **12** : 189–201, figs. 1–7. 1918.
20. **Tahara, M.** Cytological studies on Chrysanthemum. Bot. Mag. Tokyo **29** : 48–50. 1915.
21. ——. Cytologische Studien an einigen Kompositen. Jour. Coll. Sci. Imp. Univ. Tokyo **43**^{7} : 1–53. Pls. 1–4. 1921.
22. **Tangl, E.** Die Kern- und Zelltheilungen bei der Bildung des Pollens von *Hemerocallis fulva* L. Denkschr. Akad. Wiss. Wien, Math.-Naturw. Cl. **45** : Pls. 1–4. 1882.
23. **Timberlake, H. G.** The development and function of the cell plate in higher plants. Bot. Gaz. **30** : 73–99; 154–170. Pls. 8, 9. 1900.

DESCRIPTION OF PLATE XV

The photomicrograph was made with 1½ inch Professional Bausch and Lomb objective 24° and ocular 7.5 × with the plate at a distance of 42½ centimeters from the slide. The drawings were made after tracing the outline with a camera lucida. A Zeiss homogeneous immersion 2-mm. lens with aperture 1.30 was used with an 8 × ocular at a tube length of 145 mm.

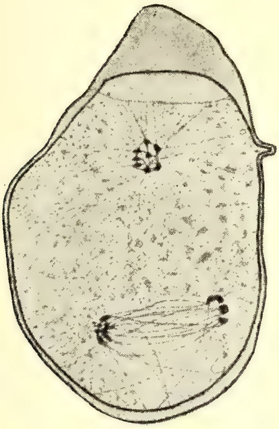
FIG. 1. Photomicrograph of median longitudinal section of an anther of *Nelumbo lutea*, showing two pollen chambers with little more than a single row of pollen mother cells disposed freely within each pollen chamber.

FIG. 2. Pollen mother cell in a very late anaphase stage showing the lateral view of one spindle and a polar view of the other. Eight chromosomes may be counted. No evidence of a cell plate across the equator of the spindle is apparent.

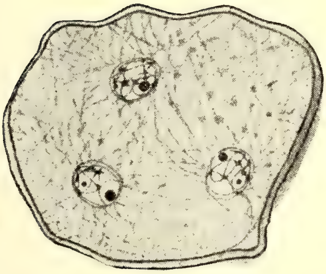
FIG. 3. A tetranucleate stage in pollen formation. The nuclei have already enlarged somewhat. A cell plate, if present, should be well formed at this stage, but there is no indication of such a structure.

FIG. 4. A late stage of quadripartition by furrowing. The cytoplasm has become progressively more and more fibrillar, and the nuclei are larger. Note the relative thinness of the furrow and its uniform width. The unequal thickening of the mother-cell wall is well shown in this figure.

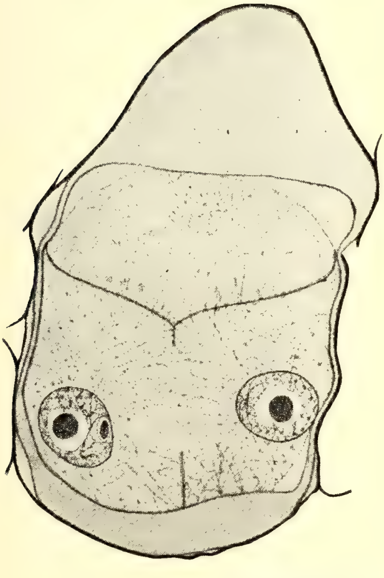
2

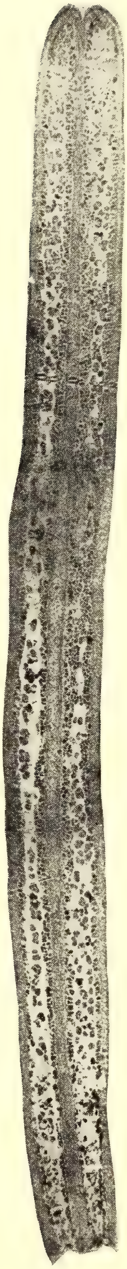


3



4





1

Farr: Cytokinesis of Nelumbo

COMPARATIVE STUDIES ON RESPIRATION XXII.

THE EFFECT OF LACTIC ACID ON THE RESPIRATION OF WHEAT

Edith Philip Smith

(Received for publication November 9, 1921)

Respiration in plants and animals involves a continuous series of linked reactions, of oxidative character, the end products being carbon dioxide and water. The reaction proceeds by stages, and though the actual steps may be unknown, many substances have been suggested as intermediate products. The hypothesis that the introduction into a respiring system, from without, of an excess of any one of the substances which are supposed to be intermediate stages in the metabolism, should accelerate the rate of production of carbon dioxide, forms a good basis of trial on which to test these suggested substances. On account of its frequent occurrence and apparent importance in animal metabolism, lactic acid was chosen as the subject of these experiments.

In dealing with animals, the effect of muscular action on the rate of production of carbon dioxide has to be taken into account. In plant material there are no such complications; it is easy to secure seedlings at a stage when the root system is well developed while the shoot has not yet begun to show green. With long roots and abundance of root hairs there can be no question as to the successful penetration of the reagent, which is an important point. Wheat was accordingly chosen as the material for these experiments. It was germinated with aseptic precautions, and used when the roots were about two inches long and well supplied with root hairs.

The method used for studying the respiration was that described by Osterhout,[1] using phenolsulphonphthalein as indicator. The normal rate of respiration (in distilled water) was taken as the reciprocal of the time required to change the indicator from pH 7.36 to pH 7.09, and was expressed as 100 percent. The actual time varied from 30 seconds to 1 minute, according to the age of the seedlings.

After taking the normal rate of respiration, the machine was stopped and a solution of lactic acid in distilled water was substituted for the distilled water in the flask containing the seedlings. The same volume, usually 100 cc., was used in each case. The first effect was a depression in the rate of respiration, due to the necessity of saturating with carbon dioxide the volume of fresh liquid introduced into the closed system. This "satu-

---

[1] Osterhout, W. J. V. Jour. Gen. Physiol. 1 : 17–22. 1918.

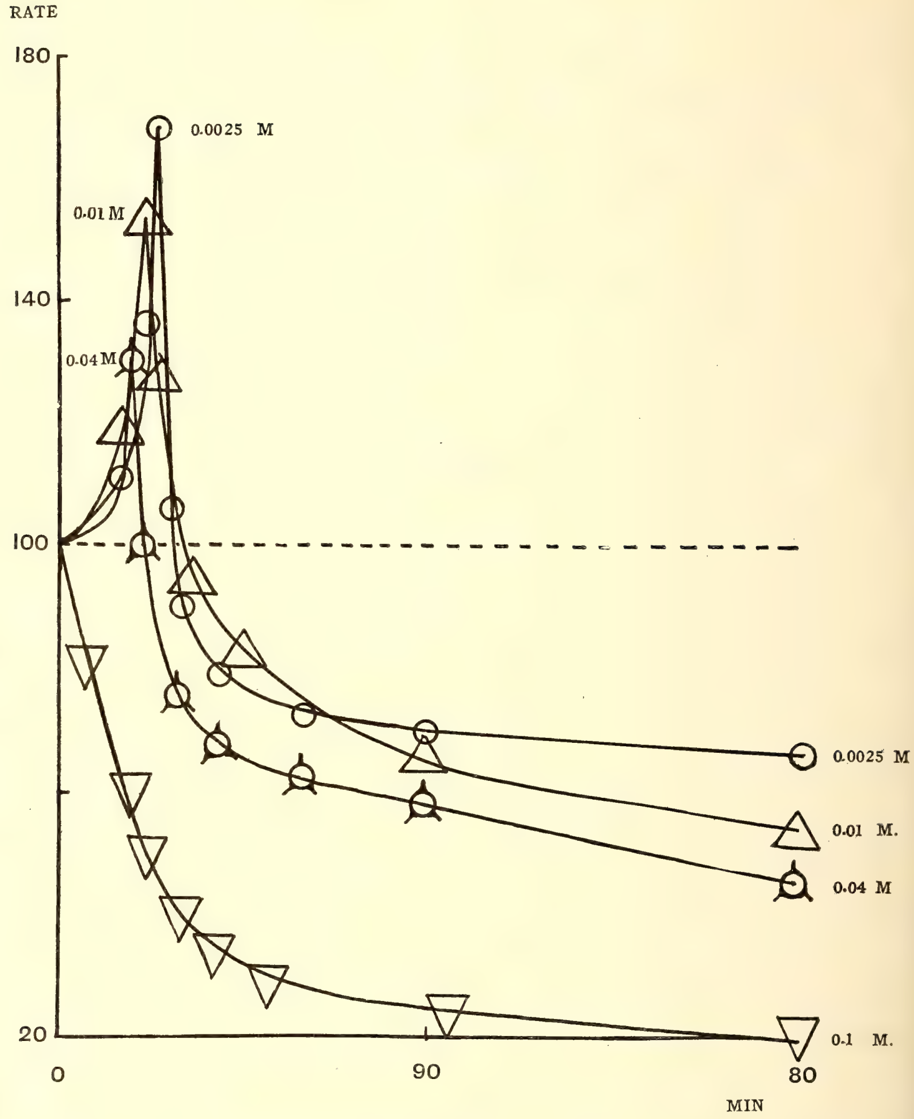


FIG. 1. Curves showing the rate of respiration of wheat seedlings (expressed as percentage of the normal rate), in lactic acid 0.0025M, 0.01M, 0.04M, and 0.1M. The normal rate (which is taken as 100 percent) is the reciprocal of the time required to change the indicator from pH 7.36 to pH 7.09 (usually from 30 to 60 seconds, according to the age of the material). The curve with 0.0025M lactic acid represents the mean of three experiments; probable error of the mean, less than 5 percent of the mean (after the maximum, less than 2 percent of the mean). The curve with 0.01M acid is the mean of two experiments; probable error of the mean, less than 3 percent of the mean. The curve with 0.04M acid represents a single typical experiment. The curve with 0.1M is the mean of five experiments; probable error of the mean, less than 10 percent of the mean.

ration effect" was determined for distilled water for each individual experiment, and allowance was made for this in drawing the curve. In no case did this preliminary lag in distilled water endure for more than 10 minutes, and it was shown, by introducing the lactic acid into the system in a separate tube, that it had no more buffer effect than the same volume of distilled water.

The lactic acid was used in the following concentrations: 0.0025*M*, 0.005*M*, 0.01*M*, 0.02*M*, 0.04*M*, 0.1*M*, 0.2*M*, 0.5*M*, and 2*M*. This range was sufficiently close to give a good series of curves.

With lactic acid 0.0025*M*, the first effect was a rise in the rate of respiration, which reached a maximum of 168 percent in 24 minutes after beginning the exposure to the acid. In 27 minutes the rate reached normal again, and then proceeded to fall below it. In 2 hours the rate had fallen to 68 percent.

With a concentration of 0.01*M*, a maximum of 153 percent was reached in 21 minutes from the first exposure. The rate remained above normal for 30 minutes, and then fell to 61 percent in 2 hours.

With 0.04*M* lactic acid, the initial rise was smaller, and the time above normal was less. Thus, the maximum of 130 percent was reached in 18 minutes, and the rate returned to normal in 21 minutes. After 2 hours the rate was 55 percent.

With 0.1*M* lactic acid, there was no rise apparent. The rate fell steadily, at first rapidly and then more slowly, reaching 40 percent in 30 minutes, 28 percent in one hour, and 24 percent in two hours. After 4 hours the rate was reduced to 16 percent.

With intermediate strengths the results were intermediate and similar to those given. The figures represent the mean of several closely agreeing experiments.

In order to determine whether the osmotic pressure or the pH value of the lactic acid was contributing to the results obtained, experiments were made with sulphuric acid of the same pH value (about pH 3) as the 0.1*M* lactic acid. It was found that the sulphuric acid had no more effect than the same volume of distilled water. In the same way a 3 percent solution of dextrose, which was known to have a greater osmotic pressure than the 0.1*M* lactic acid, was found to have very little more effect than distilled water: and the "saturation effect" lasted only a few minutes longer. It was thus reasonably certain that the observed results were really due to some specific action of the lactic acid.

Recovery experiments were made with the 0.1*M*, 0.2*M*, and 2*M* acid. In all cases the seeds recovered completely on removal to distilled water, if sufficient time was allowed. Recovery was possible even if the rate had been reduced to 25 percent of the normal.

In these experiments there was no evidence of any permanent increase in the rate of production of carbon dioxide. This would seem to indicate that in the case of wheat seedlings lactic acid is not an important stage in the normal metabolism. This is interesting in view of its apparent importance in the animal metabolism, and further experiments on animal tissues by this method should prove valuable.

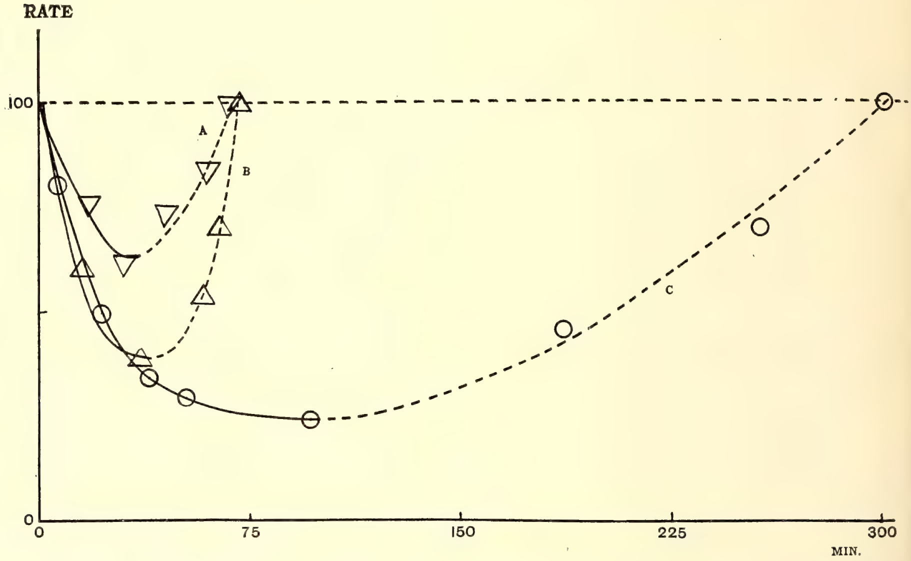


FIG. 2. Curves showing recovery from exposure to lactic acid. Normal rate as in figure 1. Solid line represents respiration in lactic acid; broken line, respiration in distilled water. Curve *A* in 0.2M acid, curves *B* and *C* in 0.1M acid. Each represents a single typical experiment.

CONCLUSIONS

1. In high dilutions, such as 0.0025M, lactic acid first accelerates and then depresses the rate of production of carbon dioxide by wheat seedlings.

2. As the concentration of the acid increases, the preliminary rise in rate becomes less marked, till a concentration is reached when the rate begins to fall at once.

3. Even if the rate has been rapidly reduced to 25 percent of the normal by 2M lactic acid, recovery is possible and appears to be complete.

4. The observed effects are due not merely to osmotic pressure or to acidity, but to some specific action of the lactic acid.

Since there is no permanent increase in the rate of production of carbon dioxide, as would be expected on the hypothesis that lactic acid is a stage in the metabolism of wheat, it may be concluded that lactic acid is not, in this case, an important intermediate substance.

LABORATORY OF PLANT PHYSIOLOGY,
HARVARD UNIVERSITY

THE EFFECT OF TRANSPIRATION ON THE ABSORPTION OF SALTS BY PLANTS

Walter C. Muenscher

(Received for publication November 22, 1921)

Various opinions have been offered regarding the relation between transpiration and the absorption of solutes. Those investigators who support the older theory maintain that the quantity of salts taken into a plant is directly proportional to the amount of water transpired, and that the quantity of water transpired is in inverse ratio to the concentration of the solution. Some workers maintain that there is no direct relation between transpiration and the absorption of salts. In other words, the rate at which salts enter the cell is independent of the rate at which water enters the cell. Others accept the theory that the water and solutes of a solution enter a plant at independent rates, but maintain that after the solutes enter the plant they move along with the water in the "transpiration stream."

A large amount of literature has appeared dealing with transpiration and water requirements, but, with few exceptions, no papers have appeared which offer any considerable data on a possible relation between transpiration and the intake of solutes. In spite of this fact, many authors have not hesitated to employ various theories regarding the relation between transpiration and the absorption of salts in explaining results which they have obtained in investigations upon transpiration and water requirements. The fact still stands, however, that a careful search through the literature reveals no conclusive data which would substantiate the correctness of any of these theories. From a theoretical standpoint it would be reasonable to assume that the entrance of water and solutes into the plant takes place independently, since they enter not through openings which would allow for mass flow but through membranes which necessitate diffusion. Actual data presented are conflicting. The writer has been carrying on a number of experiments with reference to a possible relation between transpiration and the absorption and distribution of salts in plants. The results obtained in those experiments bearing on absorption only are reported in this paper.

Historical

Some of the earlier workers varied transpiration by increasing the supply of water or mineral nutrients and determined the effect of this change upon the dry weight and ash content of the plants. Lawes (1850) reported results obtained with several legumes and cereals grown in jars of soil treated with nutrient solutions. His analyses of the tops show that when transpiration was increased sometimes the amount of ash increased and sometimes it decreased. Ilienkoff (1865) found no correlation between the quantity of water supplied and the total ash content of buckwheat plants the tops of which were analyzed after growing for a period of 67 days. Fittbogen (1873) found no significant difference in the quantity of ash found for a unit of water transpired by oat plants growing in soil in which the water varied from 20 to 60 percent of its water-holding capacity.

Thom and Holtz (1917) presented data that show that in general as the concentration of the soil solution is increased the water requirement of wheat and barley plants decreases as does also the quantity of water transpired per gram of ash found in the plant. Their table at the top of page 50 shows that the ash content in the whole plants, expressed in percentage of dry weight, is about the same regardless of the concentration of the nutrient solution in which they were grown, excepting in a very high concentration which was injurious to growth.

The effect of decreased transpiration brought about by shading has been worked on first by Schloessing (1869), and more recently by Hasselbring (1914 *a*, *b*). Schloessing found that a tobacco plant under a shaded bell jar which transpired the least also possessed the smaller dry weight and ash content. Hasselbring, also working with tobacco plants, found, on the other hand, a smaller absolute amount and percentage of ash in the plants which transpired the most. The dry weight was practically the same in plants grown in the open sunlight and in the shade. He stated that it appears, therefore, that the absorption of salts by roots is independent of the absorption of water, and that the transpiration stream does not exert an accelerating effect on the entrance of salts.

Sorauer (1880) and Wollney (1898 *a*, *b*) worked on the relation of absorption of salts and transpiration as affected by atmospheric humidity. Sorauer found that pea plants growing in a dry chamber had a slightly greater dry weight and ash content than similar plants growing in humid chambers. Wollney, working with several crop plants grown in chambers with high, medium, and low humidity, found that in general the absolute green weight and dry matter was somewhat greater in the plants grown in the more humid atmosphere. The percentage of ash and dry matter in general increased slightly with the dryness of the air. Kiesselbach (1916) found no distinct correlation between transpiration and the absolute quantity or percentage of ash or between the water requirement and ash found in corn plants grown in dry and humid greenhouses, or in different degrees of soil-moisture content. Curtis (1920) mentioned some unpublished data showing that doubling the transpiration of barley plants growing with their roots in nutrient solution has no tendency to increase salt absorption when the transpiration is increased by decreasing the atmospheric humidity.

Several authors have compared the absorption of mineral nutrients and growth by plants growing in habitats where transpiration is reduced with that by plants growing in habitats where conditions for transpiration are more favorable. Among these may be cited the work of Schimper upon halophytes, and the work of Haberlandt, Burgerstein and his co-workers, and McLean upon transpiration by plants in tropical rain forests.

Schimper (1891) pointed out that the xerophytic modifications of halophytes serve to reduce transpiration and also the absorption of salts. Although he does not say that salts enter with the water, he assumes a relation between transpiration and the absorption of salts when he states that reduced transpiration also reduces the absorption of salt and protects the plant from the danger of too much salt accumulating in the leaves. The works of Stahl (1894) and others offer better explanations for the occurrence of xerophytic adaptations among halophytes.

Haberlandt (1892) first definitely suggested that there is no relation between transpiration and the quantity of mineral nutrients absorbed from the soil. He maintained that diffusion independently of transpiration causes the movement of salts from the roots to the highest parts of plants. Transpiration is only one factor and not the important one in the movement of salts. Haberlandt presented data to show that the rate of transpiration in tropical forests is less than in central Europe. High relative humidity in spite of high temperatures reduces the transpiration in the tropics. Not all workers agree with Haberlandt. In opposition to Haberlandt, Burgerstein (1897), Stahl (1894), and Giltay (1897) claimed that the plants in the tropical rain forests transpire more than those in a temperate region such as central Europe. It is beside the question to discuss the validity of claims made by the opponents in this controversy. It will suffice here to point out that Haberlandt, who believed that the transpiration in the tropics is not higher than in central Europe, also maintained that transpiration is not necessary for the absorption of salts; while Burgerstein and his followers, who believed that the transpiration in the tropics is very high, adhered to the theory that there is a relation between transpiration and the absorption of salts. Burgerstein said that a green plant in synthesizing large quantities of organic matter needs large quantities of inorganic nutrients, and that, since these are in a very dilute solution in the soil water, the plant must take up large amounts of this solution and transpire the excess water. Since only a small amount of this water is used in synthesis, transpiration makes it possible for a plant to conduct large quantities of water and nutrients to the assimilating tissues in a short time.

McLean (1919) worked with plants of the tropical rain forests of Brazil. He found that leaves taken from the plants growing under these conditions of assumed depressed transpiration showed a higher ash content relative to the total assimilates than sun plants. This he takes to indicate that

> The absorption of mineral salts is independent at least of foliar evaporation, the most complete suppression of which is thus seen to be of only secondary importance to the plant.

Whether the suppression of foliar evaporation signifies the suppression of a water current in the axis does not appear.

The authors of several important textbooks of plant physiology—Sachs (1887), Pfeffer (1900), and Jost (1907)—state that a "transpiration current" is necessary for supplying adequate quantities of the necessary salts to the plant. These authors make statements to the effect that it is more or less obvious that it is necessary for a plant to take up larger amounts of a dilute solution than of a concentrated solution in order to supply the salts necessary for growth. None of these authors presents any new data nor even cites conclusive data which supports his conclusions. It is true, as McLean (1919) has pointed out, that the almost complete suppression of transpiration does not necessarily signify the suppression of a water current; but in this connection it should also be borne in mind that the existence of a transpiration stream in the sense of mass movement of solution in normal growing plants has yet to be demonstrated.

This brief review of a number of works which include some data or discussions of the relation of transpiration to the absorption of salts serves to indicate that the data presented and the conclusions drawn are often contradictory. It would be surprising indeed to find a group of workers obtaining the same results and arriving at the same conclusions under such a variety of methods. It might be expected that in a study of the relation between transpiration and the absorption of salts the results might differ depending upon whether transpiration was varied by changing the atmospheric humidity, light intensity, soil water, or concentration of the nutrient solution. All these factors affect transpiration, but when one varies one of these factors in endeavoring to reduce transpiration, he may also vary some other factor or factors which may become the determining factor. Perhaps a lack of consideration of the principle of limiting factors in addition to a lack of uniform methods and materials is largely responsible for some of the contradictory results presented by various investigators.

A few early investigators presented data which seemed to them to indicate that there is a relation between transpiration and the absorption of salts; others got no definite results. Teachers and investigators were inclined to accept the former results and to disregard the latter. Even the writers of many of the physiological textbooks in use today make very definite statements regarding the relation of transpiration to the absorption of salts with nothing except the fragmentary data of some of these earlier workers and speculation upon which to base their statements. Results obtained by some of the more recent workers indicate that there is not necessarily a relation between the quantity of water transpired and the quantity of salts absorbed. Perhaps other factors besides transpiration are more important in determining how large are the quantities of salts absorbed by plants.

General Outline of Experiments

The experiments which are reported in this paper were undertaken in order to obtain data which might indicate whether or not there is any relation between absorption of water and of mineral nutrients. The absorption of water was determined by measuring volumetrically the amount of water lost from the containers. The absorption of salts was determined by analyzing the plants for total ash. It is realized that the measure of ash does not represent an actual measurement of salts absorbed. However, with a uniform solution and a uniform method of analysis, for a given species the results are comparable as far as relative values are concerned. Two series of experiments, summer and winter, were conducted in the greenhouse. In the summer series the rate of transpiration was reduced by increasing atmospheric humidity and by decreasing the intensity of sunlight. In the winter series transpiration was reduced by decreasing the light intensity and also by increasing the concentration of the nutrient solution. Cultures were grown under the following conditions:

I. *Summer Series*

a. Dry chamber; standard Knop's solution[1] (.14%).
b. Humid chamber; standard Knop's solution (.14%).
c. Sunlight; standard Knop's solution (.14%).
d. Shade tent; standard Knop's solution (.14%).

II. *Winter Series*

e. Sunlight; dilute Knop's solution (.07%).
f. Shade tent; dilute Knop's solution (.07%).
g. Sunlight; concentrated Knop's solution (.28%).

Materials and Methods Used

Summer Series

The chambers used for the dry and humid series of cultures consisted of large glass cases each 140 centimeters long, 70 centimeters wide, and 110 centimeters high, placed in the middle of a greenhouse room. The air was kept in circulation continuously during the daytime by a fan in each chamber. Fresh air was pumped into the chambers by a compressor which was attached to a motor outside the chamber.

The atmospheric humidity in the humid chamber was kept high by a

---

[1] The following standard Knop's solution was employed as the basis for making up the nutrient solutions:

$Ca(NO_3)_2$	0.8 gram
KNO_3	0.2 gram
KH_2PO_4	0.2 gram
$MgSO_4$	0.2 gram
$FePO_4$	trace
Distilled water	1,000 cc.
Total	1.4 grams

system of twelve Livingston's porous-cup atmometers which were fed from an elevated water tank. The bottom of the chamber was also covered by two large flat trays of water. Under these conditions it was possible to keep the relative humidity between 70 and 100 percent. The temperature varied from 15° to 31° C. during the daytime, usually averaging about 25° C. The atmospheric humidity in the dry chamber was kept low by the use of anhydrous calcium chloride. Under these conditions it was possible to keep the relative humidity between 30 and 60 percent. The temperature variations in the dry chamber were about the same as in the humid chamber, but often the temperature was slightly higher in the former. The evaporating power of the air was determined by standardized Livingston's dark porous-cup atmometers. The average quantity of water lost per day for seven days was 13 cubic centimeters in the dry chamber as compared with 6 cubic centimeters in the humid chamber.

The cultures growing in the sunlight were placed about 20 centimeters apart on a greenhouse bench. The relative humidity under these conditions varied from 25 to 60 percent. The temperature was usually between 22° and 26° C., but the extremes were 14° and 30° C. The cultures grown in the shade were on the same greenhouse bench, but were covered with a tent of the same size and shape as the dry and humid chambers, made of two layers of cheesecloth, so as to reduce the sunlight. Within this tent the relative humidity was usually about 5 percent higher than in the open sunlight. The temperature was usually from 1 to 5 degrees lower than in the open sunlight. The average daily evaporation from standardized atmometers was 29 cubic centimeters in the sunlight and 17 cubic centimeters in the shade.

Barley (*Hordeum vulgare* L.) was used in this experiment. In order to avoid as far as possible error due to individual variation, seed from a pure line of barley was obtained from the department of plant breeding, Cornell University. The seeds, selected for uniformity in size and shape, were sterilized by formalin treatment, and germinated. When the roots were about four centimeters long the seedlings were planted in culture jars. The culture jars used throughout these experiments were quart fruit jars of the "Improved Mason" brand which were covered with black paper. Four seedlings were planted in each culture jar. After standing on a greenhouse bench for one week, those cultures which contained four healthy plants were divided into four similar lots of 28 cultures and placed under the following conditions:

a. Dry chamber
b. Humid chamber
c. Sunlight
d. Shade tent

These cultures were grown for five weeks, August 4 to September 8, 1920. During this time the water lost by transpiration was replaced with distilled water every two or three days, and the solution was changed every fifth day. At the end of five weeks all cultures were taken down and the green weight, dry weight, and ash weight were determined for the tops and roots of each culture. After the dry weights had been determined, the tops and roots were incinerated in an electric furnace to determine the total ash content. The ashing was made at a low red heat for several hours. This furnace carried a load of eight crucibles at a time. In order to reduce the error due to the method of ashing, each load consisted of one crucible containing the tops and another containing the roots of one culture from each of the four conditions under which the cultures were grown.

Winter Series

The materials and methods employed in the winter series were the same as those employed in the summer series. The plants used in this series came from the same lot of seed as was employed for the summer series. Two solutions were used, one a dilute Knop's solution (0.07 percent) and the other a concentrated Knop's solution (0.28 percent). After these cultures had remained upon the greenhouse bench for one week, all those which did not contain four healthy plants were discarded, and the rest were divided into three groups and placed under the following conditions:

e. Sunlight; dilute Knop's solution (0.07%)
f. Shade tent; dilute Knop's solution (0.07%)
g. Sunlight; concentrated Knop's solution (0.28%)

These cultures were grown for five weeks, from January 19 to February 24, 1921. During this time the water lost by transpiration was replaced with distilled water every two or three days, and the solution was changed every fifth day. At the end of five weeks the cultures were all taken down, and the green weight, dry weight, and ash weight were determined for the tops and roots of each culture.

DATA AND DISCUSSION

Summer Series

Dry–Humid Cultures

Table 1 presents a summary of the data obtained from the cultures in which transpiration was varied by changing the atmospheric humidity of the chambers in which they were grown. The plants in the humid chamber were slightly taller and their leaves were slightly longer than those in the dry chamber. The roots of the plants grown in the dry chamber were on the average about six centimeters longer and branched more profusely than those of the plants grown in the humid chamber. The total green weight was slightly greater in the plants grown in the humid atmosphere, probably because of the greater quantity of water in their tissues. The total dry weight was slightly greater in the plants grown in the dry atmosphere. The total ash content was only slightly greater, 147 milligrams per culture in the plants grown in the dry atmosphere as compared with 135 milligrams per culture in the plants grown in the humid atmosphere. The total ash expressed as percentage of dry weight was only about five percent less (ratio of dry to humid = 100 : 94.7) in the plants grown in the humid chamber than in those grown in the dry chamber. The ash, expressed as percentage of green weight, was about fourteen percent less (ratio of dry to humid = 100 : 86.4) in the plants grown in the humid chamber.

TABLE 1. *Relation of Ash Content in Barley Plants to the Amount of Transpiration as Affected by a Difference in Atmospheric Humidity. Summer Series. Plants Grown 5 Weeks (August 4 to September 8, 1920)*

	Dry Chamber			Humid Chamber		
	Tops	Roots	Plants	Tops	Roots	Plants
No. of cultures averaged			25			25
Green weight per culture (grams)	6.5	2.2	8.7	7.206	2.067	9.270
Dry weight per culture (grams)	.60	.11	.71	.5854	.1068	.6922
Total ash content per culture (grams)	.125	.022	.147	.116	.019	.135
Ash content (percentage of green weight)	1.92	1.00	1.69	1.61	.90	1.46
Ash content (percentage of dry weight)	20.7	20.02	20.63	19.63	18.07	19.54
Total water transpired (cc.)			350			170
Water used per gram dry matter (cc.)			492.96			245.59
Water used per gram ash content (cc.)			2,380.95			1,259.25

The data show that by increasing the atmospheric humidity the quantity of water transpired was reduced from 350 cubic centimeters to 170 cubic centimeters per culture for the period of five weeks. This reduction in transpiration also correspondingly reduced the water requirement from 492 to 245. The quantity of water transpired per gram of ash content found in the plants was also reduced to approximately one half when transpiration was reduced. These data seem to check with those reported by Hasselbring (1914 *a*), Kiesselbach (1916), McLean (1919), and Curtis (1920), indicating that there is no direct relation between transpiration and the ash content in plants.

The fact that the absolute quantity or percentage of ash is reduced but slightly when transpiration is reduced to less than one half seems significant evidence against the theory that there is a direct relation between transpiration and the absorption of salts. Even the slightly greater ash content of the plants in the dry chamber seems to be determined by some factor other than the amount of water absorbed, namely food supply, which will be discussed later. If the salts in the solution enter the plant with the water and the entrance of the water is determined largely by the rate at which it is transpired, then, for a given concentration of salts, the greater the quantity of water transpired the greater will be the amount of salts brought in by the water absorbed. Burgerstein (1897) stated that a growing plant must take up large quantities of water to supply it with the necessary inorganic elements. Sorauer (1880) and Thom and Holtz (1917) suggested that the greater the concentration of the nutrient solution the smaller the quantity of it necessary to supply the plant with the necessary amounts of nutrient salts. Sachs (1887), Pfeffer (1900), and Jost (1907) implied a direct relation between transpiration and the absorption of salts. The data presented in table 1 do not bear out any direct relation between transpiration and the absorption of salts in barley plants grown in water cultures. On the contrary, the data indicate that the salts enter the plant independently of the rate of transpiration.

Light–Shade Cultures

Table 2 presents a summary of the data obtained from the cultures in which transpiration was reduced by shading. The shaded plants had slightly taller tops but were more slender and had fewer leaves than the plants growing in the open sunlight. The shaded plants stooled very little while the plants growing in the sunlight all stooled profusely. The roots of the shaded plants were much shorter and had fewer branches than those grown in the sunlight. The total green weight, dry weight, and ash weight were reduced to less than one half in the shaded plants. The shading not only reduced transpiration, but also reduced the photosynthetic activity of

TABLE 2. *Relation of Ash Content in Barley Plants to the Amount of Transpiration as Affected by a Difference in Light Intensity. Summer Series. Plants Grown 5 Weeks (August 4 to September 8, 1920)*

	Light			Shade		
	Tops	Roots	Plants	Tops	Roots	Plants
No. of cultures averaged			25			24
Green weight per culture (grams)	12.42	4.41	16.82	5.37	1.83	7.20
Dry weight per culture (grams)	1.235	.2897	1.525	.509	.085	.5944
Total ash content per culture (grams)	.2335	.0885	.322	.1036	.0173	.1209
Ash content (percentage of green weight)	1.88	2.01	1.91	1.93	.95	1.68
Ash content (percentage of dry weight)	18.91	30.55	21.13	20.34	20.35	20.34
Total water transpired (cc.)			833			400
Water used per gram dry matter (cc.)			546.24			672.95
Water used per gram ash content (cc.)			2,586.95			3,308.52

the plants. This limited the amount of food available for growth, and as a result of checked growth smaller quantities of inorganic nutrients were used. The total ash expressed as percentage of dry weight was only about four percent less (ratio, light to shade = 100 : 96.3) in the shaded plants. Expressed as percentage of green weight the ash was about twelve percent less (ratio, light to shade = 100 : 87.9) in the shaded than in the unshaded plants.

The total transpiration per culture for the period of five weeks was 833 cubic centimeters in the unshaded as compared with 400 cubic centimeters in the shaded cultures. The water requirement, contrary to the common idea that this always increases under conditions favoring high transpiration, decreased from 672 in the shaded cultures to 546 in the unshaded cultures which actually transpired more than twice as much as the shaded plants. The amount of water transpired per gram of ash was considerably less in the unshaded plants than in the shaded ones. This is just the opposite of the results obtained when transpiration was doubled by decreasing the atmospheric humidity, which doubled both the water requirement and the water used per gram of ash (table 1).

These data agree with those of Schloessing (1869) who found that a tobacco plant grown under a shaded bell jar had a smaller total ash content and a smaller dry weight than plants grown in the open. When the plant was shaded, not only was transpiration reduced, but the amount of available food was also limited, growth was checked, and the plant utilized smaller quantities of inorganic nutrients. Hasselbring (1914 *a*) did not find a reduction in total ash content or dry matter by shading tobacco plants. It is probable that light was not reduced enough to become a limiting factor for tobacco plants under the conditions of his experiment in Cuba. His shaded plants were much larger than those grown in the open, and perhaps the increased photosynthetic area was enough to offset any reduction in the rate of photosynthesis due to shading. In the experiment reported here light was a limiting factor.

The data presented in table 2 show that, when transpiration was reduced by shading, the total ash content was also reduced. This might lead one to infer that there exists a relation between transpiration and the absorption of salts. It must be remembered, however, that, when transpiration was reduced by shading, the photosynthetic activity was also reduced at the same time, as is shown by the fact that both green and dry weights of the shaded plants were less than one half as great as those of the plants that were not shaded. If checking the transpiration alone were responsible for the reduced ash absorption, then the results of the dry–humid cultures presented in table 1 should correspond with the results of the light–shade cultures in table 2. These data show that when transpiration is reduced to one half by increasing the humidity, the total dry matter and ash were reduced only slightly and the water requirement and the water used per gram of ash were reduced to approximately one half (table 1). When transpiration was reduced to one half by shading, the total dry matter and ash were reduced to considerably less than one half while the water requirement and the water used per gram of ash were increased considerably (table 2).

These results seem to indicate that growth, as limited by food supply, rather than the rate of transpiration, determines the rate of absorption and total absorption of salts by plants. This view is strengthened by the fact that, in these experiments, the average percentage of ash expressed in terms of dry weight in whole barley plants of the same age is about the same regardless of whether transpiration was reduced by increasing atmospheric humidity or by decreasing the light intensity by shading. Table 3 presents a summary of the percentages, with probable errors,[2] of ash based upon the dry weights of tops, roots, and total plants, of the cultures grown under the various conditions in the summer series. Table 4 presents a summary of the percentages with probable errors of ash based upon green weights of the same cultures.

There is no good criterion for measuring growth under all conditions. If total green weight is used, one must consider the variation in the water content in the tissues of the plants growing under various conditions. Data presented in tables 1 and 2 indicate that the water content of the plants grown in the humid atmosphere or in the shade is much higher than in plants grown in a dry atmosphere or in the open sunlight. This probably explains the variation in ash content expressed as percentage of total green weight. Total dry weight seems to be a more satisfactory criterion for measuring growth in plants where large quantities of storage products are not formed. Table 3 shows a close relation between the weight of dry matter and ash content in barley plants regardless of the quantity of transpiration. The variation in the average percentage of ash for the cultures grown under various conditions is only about five percent of the total ash. This shows a remarkable constancy in the percentage of ash in dry weight when compared with the great variation in the quantity of water absorbed per unit of ash content of the plants under the various conditions of this experiment.

TABLE 3. *Comparison of the Average Percentage of Ash Based upon the Dry Weight of Tops, Roots, and Total Plants (Averages of cultures in tables 1 and 2)*

Set of Cultures	Percentage of Ash in Tops	Percentage of Ash in Roots	Percentage of Ash in Plants
Dry chamber	20.74 ± .027	20.02 ± .020	20.63 ± .024
Humid chamber	19.63 ± .026	18.07 ± .030	19.54 ± .013
Light	18.91 ± .017	30.55 ± .043	21.13 ± .018
Shade	20.34 ± .027	20.35 ± .048	20.34 ± .024

---

[2] For the calculation of probable errors Bessel's formula was used.

TABLE 4. *Comparison of the Average Percentage of Ash Based upon the Green Weight of Tops, Roots, and Total Plants (Averages of cultures in tables 1 and 2)*

Set of Cultures	Percentage of Ash in Tops	Percentage of Ash in Roots	Percentage of Ash in Plants
Dry chamber	1.92 ± .030	1.00 ± .024	1.69 ± .026
Humid chamber	1.61 ± .022	.93 ± .024	1.46 ± .016
Light	1.88 ± .028	2.01 ± .046	1.91 ± .019
Shade	1.93 ± .031	.95 ± .036	1.68 ± .026

Winter Series

Light–Shade Cultures

A series of cultures was set up during the winter in order to check the results obtained by shading plants in the summer experiment. Table 5 presents a summary of the data obtained. The data are self-explanatory and check very closely with the light–shade cultures of the summer series. The absolute values for green weight, dry weight, and ash weight are slightly lower throughout, but relatively the results duplicate those of the summer series. The water requirement and the quantity of water used per gram of ash content were increased considerably in the plants growing in the shade under conditions of low transpiration. This relation holds not only between the shaded and unshaded plants within the summer and winter series, but also between the summer and winter series. The plants growing on an exposed greenhouse bench and in a shade tent in winter receive much less sunlight than plants growing under similar conditions in the summer.

TABLE 5. *Relation of Ash Content in Barley Plants to the Amount of Transpiration as Affected by a Difference in Light Intensity. Winter Series. Plants Grown 5 Weeks (January 19 to February 24, 1921)*

	Light			Shade		
	Tops	Roots	Plants	Tops	Roots	Plants
No. of cultures averaged			12			11
Green weight per culture (grams)	6.97	3.01	9.98	4.34	1.75	6.09
Dry weight per culture (grams)	.765	.150	.915	.408	.052	.460
Total ash content per culture (grams)	.151	.031	.182	.078	.009	.087
Ash content (percentage of green weight)	2.16	1.02	1.82	1.80	0.51	1.43
Ash content (percentage of dry weight)	19.70	20.51	19.83	19.56	17.21	19.30
Total water transpired (cc.)			659.6			382.7
Water used per gram dry matter (cc.)			720.87			831.95
Water used per gram ash content (cc.)			3,624.17			4,398.85

This probably explains the increase in water requirement and in quantity of water used per gram of ash content in the plants of the winter series.

It is true that the concentration of the solution used in the winter series was only one half as great as in the summer series. The question might be raised as to whether in this case the dilute solution, rather than shading, might cause the increase in water requirement. The following experiment shows that this is not the case.

Dilute–Concentrated Solution Cultures

Table 6 compares the data of the cultures grown in a concentrated solution with similar cultures grown in a dilute soulution under the same illumination. The plants grown in a dilute solution have slightly higher actual green weight, dry weight, and ash weight than those grown in the concentrated solution. It is possible that a concentration of 0.28 percent may have been somewhat injurious to barley. The plants grown in a concentrated solution have even a slightly higher water requirement and use a greater quantity of water per gram of ash in the winter than the plants grown in a dilute solution in the summer. It appears, therefore, that the reduced sunlight rather than the reduced concentration of the solution is largely responsible for the increased water requirement in the cultures of the winter series, even if the actual transpiration is decreased considerably by shading.

TABLE 6. *Relation of Ash Content in Barley Plants to the Amount of Transpiration as Affected by a Difference in Concentration of Nutrient Solution. Winter Series. Plants Grown 5 Weeks (January 19 to February 24, 1921)*

	Concentrated Solution			Dilute Solution		
	Tops	Roots	Plants	Tops	Roots	Plants
No. of cultures averaged			17			12
Green weight per culture (grams)	4.92	2.5	7.42	6.97	3.01	9.98
Dry weight per culture (grams)	.662	.120	.782	.765	.150	.915
Total ash content per culture (grams)	.134	.029	.163	.151	.031	.182
Ash content (percentage of green weight)	2.72	1.16	2.20	2.16	1.02	1.82
Ash content (percentage of dry weight)	20.27	24.17	20.84	19.70	20.51	19.83
Total water transpired (cc.)			431.56			659.6
Water used per gram dry matter (cc.)			551.87			720.87
Water used per gram ash content (cc.)			2,647.61			3,624.17

Table 7 presents the percentages of ash in the tops, roots, and total plants expressed as percentages of dry weight and green weight for all cultures grown under the various conditions of the winter series. When these data are compared with the data from the summer series in tables 3 and 4, it will be noted that the percentage of ash expressed as percentage of dry weight usually varies less than five percent between the high and low transpiring plants under the various conditions under which the plants were grown. When the ash content is expressed as percentage of green weight, the variation is from 12 to 22 percent between the high- and low-transpiring plants.

Table 7. *Comparison of the Average Percentage of Ash in Tops, Roots, and Total Plants (Averages of cultures in tables 5 and 6)*

Percentage of Ash Based upon Dry Weights

Set of Cultures	Percentage of Ash in Tops	Percentage of Ash in Roots	Percentage of Ash in Plants
Conc. solution in light	20.27 ± .031	24.17 ± .046	20.84 ± .028
Dilute solution in light	19.70 ± .015	20.51 ± .026	19.83 ± .013
Dilute solution in shade	19.56 ± .035	17.21 ± .060	19.30 ± .029

Percentage of Ash Based upon Green Weights

Set of Cultures	Percentage of Ash in Tops	Percentage of Ash in Roots	Percentage of Ash in Plants
Conc. solution in light	2.72 ± .056	1.16 ± .038	2.20 ± .034
Dilute solution in light	2.16 ± .043	1.02 ± .039	1.82 ± .030
Dilute solution in shade	1.80 ± .038	0.51 ± .019	1.43 ± .033

Those who maintain that the salts enter and move within the plant with the water might say that, under conditions of low transpiration especially, a dilute solution entering a plant is not sufficient to supply all the salts that it needs, and that therefore it absorbs additional salts from the solution in which it grows to supply its needs. On the other hand, the plant which transpires freely would absorb large quantities of solution in which are taken up all the salts needed by the plant. Under such conditions low- and high-transpiring plants might have the same ash content. Needless to say, such a teleological explanation is worthless.

Table 8 was prepared to determine whether the salts available in the solutions in which the plants were grown might limit the amounts entering the plants under any of the conditions under which the plants were grown. The first column gives the concentration of Knop's solution used. The second column gives the total water absorbed per culture. The third column gives the total salts absorbed as determined by the ash found.[3] The fourth column gives the ash equivalent of the solution,[4] which indicates how much ash might have been found in the plants if the salts in the solution entered with the water in which they were in solution. These data show that in every case the quantity of salts in a volume of solution equal to the volume of water absorbed and transpired was at least as great as the quantity of ash found. In every case but one, namely, in the humid cultures of the summer series, the plants took up more water than was necessary to supply the salts found, provided they all entered with the solution.

---

[3] The initial ash content of the barley grains was so small that it was not subtracted from the total ash found in order to get the total ash absorbed. Four lots of 100 uniform barley grains each were analyzed for total ash content. The following data are given in average values per single grain: Dry weight, 0.0251 g. Ash weight, 0.00067 g. Percentage of ash, 2.68.

[4] The term "ash equivalent of solution" is an arbitrary phrase here employed for designating the number of grams of total salts (NO_3 excepted) which are present in a volume of solution which is equal to the volume of water transpired per culture.

TABLE 8. *Comparison of the Average Quantity of Water Transpired with the Average Ash Content per Culture under Various Conditions*

	Conc. of Solution	Cc. of Water Transpired	Total Weight of Ash Determined	Ash Equivalent of Solution
Summer Series				
Dry	.14%	350.	.147	.2800
Humid	.14%	170.	.135	.1360
Light	.14%	833.	.322	.6640
Shade	.14%	400.	.121	.3200
Winter Series				
Light	.07%	659.6	.182	.2638
Shade	.07%	382.7	.087	.1531
Light	.28%	431.6	.163	.6906

An examination of the data presented in the above tables shows that the relation between transpiration and the absorption of salts as determined under the conditions of these experiments varies with the method by which transpiration is changed. If there is a definite relation between the quantity of water transpired and the quantity of salts absorbed, one would expect that doubling the transpiration in plants would considerably increase the absolute weight and percentage of ash in plants. This is not the case.

An examination of the data of dry weights, ash weights, and ash content expressed as percentages of dry weights presented in tables 1, 2, and 5 shows that in general an increase in dry weight is accompanied by a relatively greater increase in the ash weight. These data are brought together for comparison in table 9. In the dry–humid cultures the slightly lower dry weight of the tops, roots, and total plants of the cultures grown in the humid chamber is in every case accompanied by a slight decrease not only in absolute quantity but also in the percentage of ash. This same relation between increase in dry weight and ash is found between the light–shade cultures of the winter series. In the light–shade cultures of the summer series a pronounced increase in the dry weight of the tops is also accompanied by an increase in total ash weight, but the percentage of ash is decreased from 20.34 to 18.91 percent. The percentage of ash in the roots is increased from 20.35 to 30.55 percent when the total dry weight and ash are increased. This increase in the percentage of ash in the roots was more than enough to balance the decrease in the tops, so that the percentage of ash in the whole plants was still slightly greater in the plants having the greater dry weight.

This slightly greater relative increase in the ash weight than in the dry weight seems to indicate that when a slightly greater quantity of food is available it is used for additional growth, perhaps largely in building up additional protoplasm. The addition of protoplasm, which is relatively higher in salts than non-protoplasmic structures, might increase the percentage of ash slightly. If, on the other hand, food is produced in excess, as was evidently the case in the tops of the plants grown in the sunlight, a point is soon reached at which the utilization of foods and inorganic salts in the building of protoplasm is limited. The surplus food may then be used in the building of cell-wall material, or it may be stored in some other form. Since cell walls and storage products in plants are usually low in ash content, it is evident that any great increase in the production of these over the production of protoplasm would lower the percentage of ash in the dry matter.

TABLE 9. *Relation between the Increase in Dry Weight and Ash in Barley Plants*

		Dry Weight in Grams		Ash Weight in Grams		Percentage of Ash		Difference in Percentage of Ash
		Dry	Humid	Dry	Humid	Dry	Humid	
Dry–Humid... Summer Series (From table 1).......	Tops.	.60	.5854	.125	.116	20.74	19.63	1.11 ± .037
	Roots.	.11	.1068	.022	.019	20.02	18.07	1.95 ± .035
	Plants.	.71	.6922	.147	.135	20.63	19.54	1.09 ± .027
		Light	Shade	Light	Shade	Light	Shade	
Light–Shade.. Summer Series (From table 2).......	Tops..	1.235	.509	.2335	.1036	18.91	20.34	− 1.43 ± .032
	Roots.	.2897	.085	.0885	.0173	30.55	20.35	10.20 ± .064
	Plants.	1.525	.594	.322	.1209	21.13	20.34	.79 ± .029
		Light	Shade	Light	Shade	Light	Shade	
Light–Shade.. Winter Series.. (From table 5).......	Tops..	.765	.408	.151	.078	19.70	19.56	.14 ± .034
	Roots.	.150	.052	.031	.009	20.51	17.21	3.30 ± .053
	Plants.	.915	.460	.182	.087	19.83	19.30	.53 ± .031

The last column of table 9 presents the differences in the percentages of ash in the tops, roots, and total plants between the high- and low-transpiring plants. In every case the relative increase of ash was higher in the roots than in the tops. Those roots showing the greatest relative increase in ash content are those which also show the greatest increase in dry matter.

This indicates a possible relation between the food supplied by the tops to the roots and the utilization of inorganic salts in root growth. The plants which were not shaded and had a greater food supply also had a relatively greater ash content in their roots. Table 10 presents the ratio of roots to tops for all cultures. The root-top ratio of dry weights increases in both series of the light–shade cultures where the food supply is increased by increased photosynthetic activity.

TABLE 10. *Ratio of Roots to Tops under all Conditions under which Cultures were Grown*

$\frac{\text{Td}}{\text{Rd}} = \frac{\text{Dry weight of tops}}{\text{Dry weight of roots}}$ $\frac{\text{Tg}}{\text{Rg}} = \frac{\text{Green weight of tops}}{\text{Green weight of roots}}$

Summer Series		$\frac{\text{Td}}{\text{Rd}}$	$\frac{\text{Tg}}{\text{Rg}}$
Dry chamber	(0.14% solution)	5·4	2.9
Humid chamber	(0.14% solution)	5·5	3·5
Sunlight	(0.14% solution)	4.2	2.8
Shade tent	(0.14% solution)	6.0	2.9
Winter Series			
Sunlight	(0.07% solution)	5.1	2.3
Shade tent	(0.07% solution)	7.8	2.4
Sunlight	(0.28% solution)	5·5	1.9

If only one of the two above-described series of cultures were used, and the results obtained were attributed to only one factor, namely, a variation in transpiration, one might conclude either that there is a relation between transpiration and the quantity of salts absorbed or that there is not, depending upon which series of cultures one happened to choose. As a matter of fact, when transpiration was reduced by shading, the photosynthetic activity of the leaves was also reduced, thus reducing the food available for growth. With a reduction in growth, smaller quantities of mineral nutrients are used and therefore inward diffusion is slower. This probably accounts for the greater difference in absolute ash content between the plants grown in the sun and in the shade as compared with the slight difference between the plants grown in the dry and humid chambers. The fact that the percentage of ash, based upon dry weight of the whole plants, varies but slightly in the plants grown in dry or humid atmosphere or in light or shade bears out the indication that in these cultures there is a relation between growth and the absorption of essential salts, regardless of the rate of transpiration.

SUMMARY

1. All plants used in the experiments reported in this paper came from a pure line of barley. The cultures were grown for five weeks in Knop's solution in quart jars under conditions of high and low transpiration.

2. The transpiration rate was reduced by (1) increasing the atmospheric humidity, (2) reducing the light intensity, (3) increasing the concentration of the nutrient solution.

3. The absolute weight of green material, dry matter, and ash was determined for the tops, roots, and total plants in each culture. The ash content was expressed in percentage of green weight and dry weight.

4. The effects of reduced transpiration upon the total ash content of the plants used in these experiments depended upon how transpiration was reduced.

5. Under the conditions of these experiments, with a uniform concentration of nutrient solution, the total ash content of barley plants varied but slightly even though the quantity of water transpired was reduced to less than one half by increasing the atmospheric humidity. On the other hand, in plants in which the transpiration was reduced to less than one half by shading and the photosynthetic activity was also reduced, thus reducing the available food, the total ash content was also correspondingly reduced. When the total transpiration was reduced by increasing the concentration of the nutrient solution, the total ash content was only slightly reduced.

6. The ash content expressed in percentage of total dry weight of the whole plants varied but slightly, regardless of whether the plants were grown under conditions of high or of low transpiration and irrespective of how transpiration was reduced.

7. These results do not support the theory that transpiration has an important rôle in supplying plants with nutrient salts. The results of this investigation seem to indicate that, there being no other limiting factor, the amount of food available which would allow for growth, in which process nutrient salts are used, is an important factor in determining in how large quantities or how rapidly the essential salts enter the plant. Analyses for ash content indicate that there is little or no relation between transpiration and the absorption of salts in barley plants.

This investigation was suggested by and conducted under the direction of Professor O. F. Curtis, to whom the writer is indebted for helpful advice and a constant interest shown throughout its progress.

Laboratory of Plant Physiology,
Cornell University

LITERATURE CITED

Burgerstein, A. Ueber die Transpirationsgrösse von Pflanzen feuchter Tropengebiete. Ber. Deutsch. Bot. Ges. **15** : 154–165. 1897.

Curtis, O. F. The upward translocation of foods in woody plants I. Tissues concerned in translocation. Amer. Jour. Bot. **7** : 101–124. 1920.

Fittbogen, J. Untersuchungen über das für eine normale Production der Haferpflanze notwendige Minimum von Bodenfeuchtigkeit sowie über die Aufnahme von Bestandteilen des Bodens bei verschiedenen Wassergehalt desselben. Landw. Jahrb. **2** : 252–371. 1873.

Giltay, E. Vergleichende Studien über die Stärke der Transpiration in den Tropen und in mitteleuropäischen Klime. Jahrb. Wiss. Bot. **30** : 615–644. 1897.

Haberlandt, G. Anatomisch-physiologische Untersuchungen über das tropische Laubblatt. Sitzungsber. Akad. Wiss. Wien (Math.-Naturw. Kl.) I, **101** : 785–816. 1892.

——. Ueber die Grösse der Transpiration im feuchten Tropenklima. Jahrb. Wiss. Bot. **31** : 273–288. 1897.

Hasselbring, H. Relation between the transpiration stream and absorption of salts. Bot. Gaz. **57** : 72, 73. 1914 *a*.

——. The effect of shading on transpiration and assimilation of the tobacco plant in Cuba. Bot. Gaz. **57** : 257–286. 1914 *b*.

Ilienkoff, P. A. Einige Versuche zur Bestimmung des Einflusses welchen die Bodenfeuchtigkeit auf die Vegetation ausübt. Ann. Chem. Pharm. **136** : 160–165. 1865.

Jost, L. Lectures on plant physiology (English ed.). Pp. 564. Oxford, 1907.

Kiesselbach, T. A. Transpiration as a factor in crop production. Nebr. Agr. Exp. Sta. Res. Bull. **6**. Pp. 214. 1916.

Lawes, J. B. Experimental investigation into the amount of water given off by plants during their growth; especially in relation to the fixation and source of their various constituents. Jour. Hort. Soc. London **5** : 38–63. 1850.

McLean, R. C. Studies in the ecology of tropical rain-forest: with special reference to the forests of South Brazil. Jour. Ecol. **7** : 5–54; 121–172. 1919.

Pfeffer, W. Plant physiology (English ed.), vol. 1. Pp. 632. 1900.

Sachs, J. Lectures on the physiology of plants (English ed.). Pp. 836. 1887.

Schimper, A. F. W. Die indomalayische Strandflora. Bot. Mitth. aus den Tropen **3** : 9–30. 1891.

Schloessing, T. Tabac sous cloche et a l'air libre. Ann. Sci. Nat. Bot. V, **10** : 366–369. 1869.

Sorauer, P. Studien über Verdunstung. Agr. Physik **3** : 351–490. 1880.

Stahl, E. Einige Versuche über Transpiration und Assimilation. Bot. Zeit. **52** : 117–146. 1894.

Thom, C. C., and Holtz, H. F. Factors influencing the water requirements of plants. Wash. Agr. Exp. Sta. Bull. 146. Pp. 64. 1917.

Wollney, W. Einfluss der Luftfeuchtigkeit auf das Wachstum der Pflanzen. Inaug. Diss. Pp. 43. Halle, 1898 *a*.

——. Untersuchungen über den Einfluss der Luftfeuchtigkeit auf das Wachstum. Bot. Centralbl. **76** : 249. 1898 *b*.

PRAIRIE INCLUSIONS IN THE DECIDUOUS FOREST CLIMAX

Herbert C. Hanson

(Received for publication November 25, 1921)

Introduction

In the deciduous forest of the central states small fragments of prairie frequently occur (see fig. 1). These vary in area from a few square feet to several acres, occurring usually on hillsides exposed to the south, southeast, or southwest. Shimek (5, 6, 7) has noted them in northwestern Iowa and along the Missouri River; Pammel, MacDonald, and Clark (2) along the Missouri River in Iowa; Vestal (8) in Illinois; Pool, Weaver, and Jean (3) in southeastern Nebraska, and the writer in the vicinity of Cincinnati, Ohio. During the spring and summer of 1917, near Peru, in southeastern Nebraska, the writer studied intensively two small inclusions in relation to the surrounding forest. The data collected in this study are important in explaining the occurrence of prairie inclusions.

General Description of the Region

From the broad lowlands of the Missouri River in southeastern Nebraska arise abruptly densely wooded hills and bluffs, broken by numerous valleys and ravines. The forest of this hilly region is of the red oak-hickory type. It varies greatly in width, but in the fairly level upland soon gives way to the prairie. Pound and Clements (4) and Pool, Weaver, and Jean (3) have studied and described this forest in detail. The latter have shown by instrumental and quadrat studies that the succession from grassland is through the following stages: shrub, *Quercus macrocarpa–Q. acuminata, Q. velutina, Q. rubra–Hicoria ovata*, which is dominant in fairly mesophytic situations, and the final stage, *Tilia americana–Ostrya virginiana*, which is dominant on the moister slopes and in ravines.

A striking feature of this hilly forest region is that often on the sides and tops of the steep slopes and on the crests of ridges in the midst of the dense forest are found prairie areas varying from a few square feet to an acre or more. These fragments of prairie are usually found on south, southeast, or southwest slopes, directly exposed to the sun and to the wind which is prevailingly from the south from April 1 to October 1. The vegetation of the fragments is composed of typical prairie plants. The following grasses, forming well-defined bunches, are dominant: *Andropogon furcatus* Muhl., *Schizachyrium scoparium* (Michx.) Nash, *Sorghastrum nutans* L., and *Atheropogon curtipendulus* (Michx.) Fourn. Between the bunches are found numerous typical prairie herbs and occasionally a few shrubs.

Description of the Stations

One of these fragments was selected for detailed study (fig. 1). It was located on a very steep south slope surrounded on all sides by a dense forest composed of *Quercus rubra*, *Q. velutina* Lam., *Hicoria ovata*, *H. cordiformis* (Wang.) Britton, *Ulmus fulva* Michx., *Tilia americana* L., and others. The northern slope of the ridge on which this fragment was located was just as steep as the southern slope, but it was forested. Between the forest and the prairie was a narrow zone of shrubs, often denser than the forest itself. The chief shrubs in this zone were *Rhus glabra* L., *Cornus femina* Mill., *Zanthoxylum americanum* Mill., *Symphoricarpos symphoricarpos* (L.) MacM., *Salix humilis* Marsh., and *Toxicodendron radicans* (L.) Kuntze; and the vine *Celastrus scandens* L. was very frequently encountered. Seedlings and saplings of *Quercus macrocarpa*, *Q. muhlenbergii* Engelm., *Ulmus fulva*, and *Hicoria cordiformis* were also frequent in this zone. A narrow and poorly defined zone of *Quercus macrocarpa*, *Ulmus fulva*, and *Q. muhlenbergii* was found in places between the shrub and red oak-hickory forest. In the prairie the four bunch grasses were dominant. The principal herbs, *Petalostemum candidum* (Willd.) Michx., *P. purpureum* (Vent.) Rydb., *Lithospermum linearifolium* Goldie, *L. carolinense* (Walt.) MacM., *Astragalus missouriensis* Nutt., *Verbena stricta* Vent., *Laciniaria punctata* (Hook.) Kuntze, and *Solidago* sp., and the shrubs, *Rhus glabra* and *Toxicodendron radicans*, were scattered, especially over the lower part of the slope; *Ceanothus ovatus* Desf. and *Amorpha canescens* Pursh were scattered, and a clump of

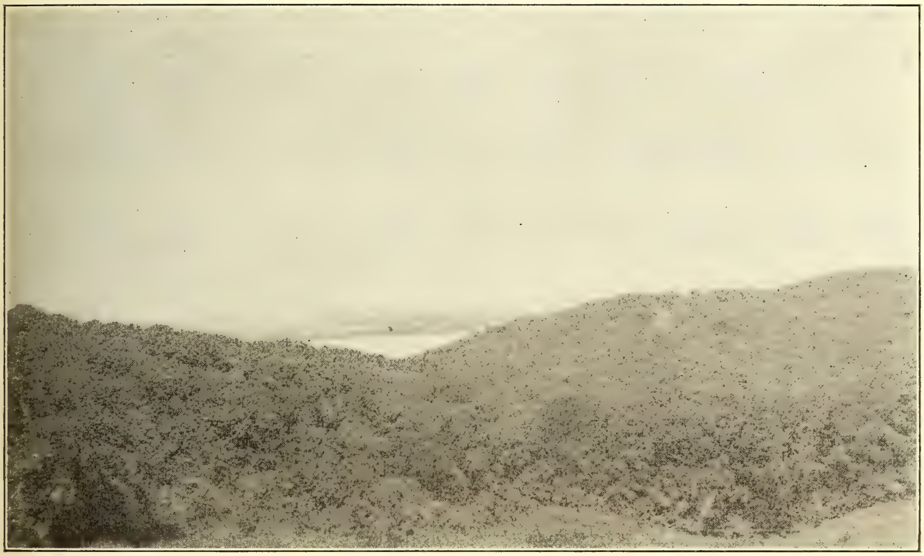


FIG. 1. Deciduous forest in southeastern Nebraska, where prairie inclusions occur.

Salix humilis occurred on the upper part of the slope. Dead bur oaks and shrubs, from one to five feet high, were scattered sparsely over the slope. It seemed probable that these were killed by a more xerophytic period following a more mesophytic one in which they had developed. A few bur-oak seedlings were found, but the small leaves, the dryness, and often the yellowish-red color of the plants showed the effects of their hard struggle.

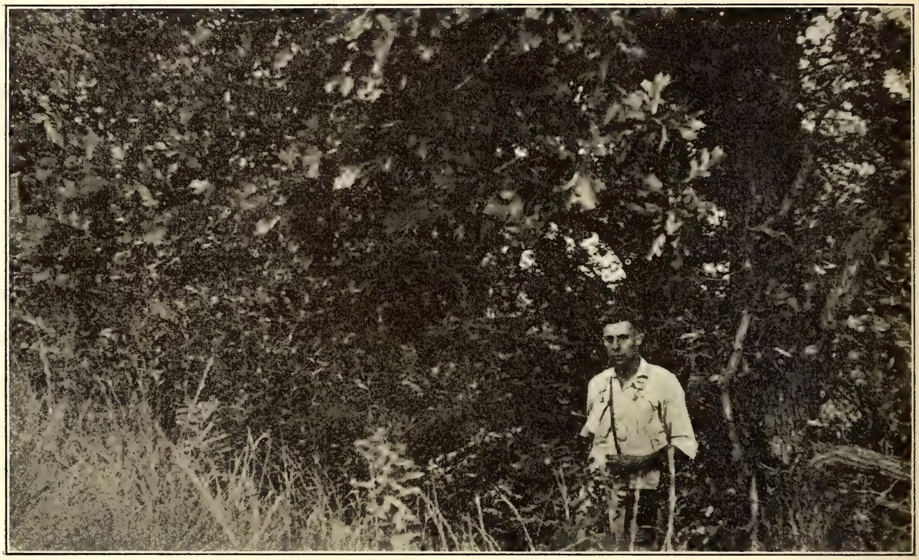


FIG. 2. Portion of a prairie inclusion showing sharp transition to forest.

Two stations were selected in the prairie fragment described above and four in the shrubs and woods surrounding this fragment. Later in the summer two more stations were selected to study another fragment. One of the prairie stations in the first remnant was located near the summit of the slope, another near the shrubs at the base, called respectively "high prairie" and "low prairie" in the following discussion. Two shrub stations were located on the gentle slope of the narrow shrub zone at the base of the remnant, and about two meters from the edge of the prairie. The shrub stations were about ten meters apart. In one, Cornus and young *Quercus macrocarpa* were dominant, while in the other *Zanthoxylum americanum*, Cornus, and *Toxicodendron radicans* were dominant. The red oak-hickory forest station was located about fifteen meters from the base of the prairie. The most abundant tree was *Quercus rubra*, while *Hicoria cordiformis* and *Ulmus fulva* occurred frequently. The luxurious undergrowth consisted of *Quercus rubra*, *Ulmus fulva*, *Cercis canadensis* L., *Hicoria cordiformis*, *Tilia americana*, *Parthenocissus quinquefolia*, *Toxicodendron radicans*, and *Symphoricarpos symphoricarpos*. The bur-oak forest station was located on the very steep north slope, just below the shrubs, which formed a zone extending for two or three meters in width from the crest. The dominant tree was *Quercus macrocarpa; Ulmus fulva*, *Hicoria cordiformis*, *Toxicodendron radicans*, *Parthenocissus quinquefolia*, and *Celastrus scandens* formed the open undergrowth. The shrubs in the zone between this forest and the prairie were *Rhus glabra*, *Cornus femina*, *Zanthoxylum americanum*, *Symphoricarpos symphoricarpos*, and *Toxicodendron radicans*.

Factor Readings

1. Evaporation

The evaporating power of the air was measured from May 10 to September 7 in the above-described stations by means of Livingston's standardized cylindrical atmometers without rain correctors. The table gives the average daily evaporation for the periods between readings and the daily average for the entire season.

TABLE 1. *Daily Amounts of Evaporation during Periods between Readings, and the Daily Average for the Season: Peru, Nebraska, 1917*

Period	High Prairie	Low Prairie	Dogwood-Bur Oak Shrub	Prickly Ash-Dogwood Shrub	Red Oak-Hickory Forest	Bur-Oak Forest
May 10–18	33.0 cc.	31.5 cc.	21.7 cc.	25.6 cc.	22.7 cc.	24.7 cc.
May 18–26	17.8	14.4	10.0	9.4	8.3	10.5
May 26–June 2	5.4	2.4	1.7	1.1	1.8	2.3
June 2–12	12.7	8.8	5.3	4.8	4.2	4.9
June 12–19	25.0	24.6	13.2	13.7	11.8	13.4
June 19–July 4	16.0	15.7	7.2	8.8	7.0	8.2
July 4–10	16.1	16.3	6.8	6.8	6.8	7.2
July 10–17	18.4	15.3	8.3	8.1	7.6	8.3
July 17–26	22.9	20.0	7.2	6.6	5.8	8.2
July 26–Aug. 2	40.6	36.2	23.7	21.4	18.2	22.0
Aug. 2–9	14.4	9.8	4.8	5.7	3.7	4.8
Aug. 9–16	18.2	14.0	5.4	5.0	4.2	5.2
Aug. 16–22	13.8	9.2	3.1	3.1	3.3	4.2
Aug. 22–31	26.1	19.1	10.6	9.9	9.7	11.2
Aug. 31–Sept. 7	20.5	16.2	6.3	7.2	5.9	8.1
Daily average May 10–Sept. 7	20.6	16.9	9.0	9.1	7.9	9.5

The table shows that the evaporation for the season was greatest in the high prairie with 20.6 cc. daily average loss, next in the low prairie with 16.9 cc., followed by bur-oak forest with 9.5 cc., shrubs with 9.1 cc., and least in the red oak-hickory forest with 7.9 cc. The greatest evaporation occurred in the high prairie because this station was more directly exposed to the sun and wind. The bur-oak forest station showed a greater evaporation loss than the shrub stations because the growth was much more open in the former, thus allowing freer circulation of the air and reducing to a less extent the light intensity. The shrub zone was so dense in many places that passage through it was almost impossible; such places did not occur in the bur-oak forest. The evaporation was lower in the low prairie than in the high prairie because it was less exposed to the sun and wind, it was in the shade later in the morning and earlier in the evening, and it retained the dew longer.

2. Soil Moisture

Soil moisture readings were taken eight times from May 18 to July 19 at depths from 0 to 10 cm. and 10 to 30 cm. in each of the six stations where the atmometers were operated. These readings, with wilting coefficients for three of the stations, are given in table 2.

At both depths, 0–10 cm. and 10–30 cm., the readings show with striking regularity that the high prairie had the lowest soil moisture, this increasing in the stations in the following order: low prairie, bur-oak forest, shrubs and red oak-hickory forest. Water was unavailable for plant growth in the high prairie at 0–10 cm. four times and at 10–30 cm. two times during the growing season. In the bur-oak station it was unavailable once at both depths. Water was available for growth in the red oak-hickory station at all readings. The low water content in the high prairie was caused by the direct exposure to sunlight and wind, and by the great amount of run-off caused by the steepness of the slope. The bur-oak forest had a lower soil water content than the shrub stations because the plant cover was less dense.

TABLE 2. *Total Water Content of the Soil at Depths of 0–10 cm. and 10–30 cm. in Various Stations at Peru, Nebraska, 1917*

Station	May 18	May 26	June 2	June 12	June 19	July 4	July 15	July 29	Average	Wilting Coefficient
0–10 cm.	%	%	%	%	%	%	%	%	%	%
High prairie	8.9	23.3	26.6	15.1	7.3	12.5	15.8	5.4	14.5	13.0
Low prairie	12.8	15.5	28.4	18.7	9.0	15.0	18.4	6.6	15.5	
Dogwood-bur oak shrub	19.8	28.3	32.9	25.9	16.8	22.6	24.0	11.4	22.7	
Prickly ash-dogwood shrub	23.1	32.1	33.9	29.9	18.7	23.5	25.9	14.5	25.2	
Red oak-hickory forest	32.7	37.1	38.5	34.0	27.9	33.0	30.9	15.7	31.2	15.5
Bur-oak forest	16.9	27.1	31.0	24.0	16.2	21.9	19.6	9.8	20.8	15.2
10–30 cm.	%	%	%	%	%	%	%	%	%	%
High prairie	13.3	19.8	23.5	18.3	11.7	16.1	12.0	6.7	15.2	11.9
Low prairie	15.3	22.3	26.2	21.5	12.7	16.3	13.2	8.5	17.0	
Dogwood-bur oak shrub	20.1	26.9	31.1	25.4	19.4	22.8	19.6	15.1	22.5	
Prickly ash-dogwood shrub	24.6	29.1	31.3	27.7	22.1	24.0	23.5	15.3	24.7	
Red oak-hickory forest	28.5	31.2	36.0	30.8	25.3	28.9	25.1	16.4	27.8	12.5
Bur-oak forest	20.1	24.8	28.5	23.1	18.7	21.8	17.2	11.7	20.7	12.5

Discussion

The maintenance of prairie inclusions against invasion by the shrub and the forest by which they are surrounded has been discussed by a few writers. Shimek (7) states that the chief factor is the exposure to evaporation as determined by temperature, wind, and topography. He believes that the "determining causes of relative prairie and forest distribution evidently lie in the atmosphere rather than in the soil" (7, p. 24), and that prairie fire was an effect rather than a cause. Vestal (8, pp. 122, 123) says:

> The essential condition is the great insolation and exposure to the dry summer winds from the south and southwest, making for local xerophytism. This is apparently a static rather than a dynamic feature of the environment, since both habitat and xerophytic vegetation may persist indefinitely, even though there is a slow lateral migration as the valley widens. It is probable that the dryness occasioned by the slope to the south is in most places not in itself sufficient to preserve the prairie from forest encroachment, for forest is able to establish itself in quite xerophytic habitats in the vicinity, and has in fact done so over most of the south-facing ravine slopes. Other physical factors aid in the original exposure afforded by direction of slope. One is instability of surface, due partly to steepness, partly to the meagerness of protection against erosion afforded by the open and sparse ground-cover. Others are accidental and artificial factors which destroy or check forest growth, such as fire, cutting, grazing, and trampling. These operate in places only temporarily, but in other places recur frequently enough to permit the continued existence within the forest of small but rather numerous patches of prairie, with more or less shifting boundaries, wherever the basic condition of southward exposure is fairly extensive.

Pool, Weaver, and Jean (3), in studying the vegetation in the vicinity of Peru, Nebraska, collected considerable factor data on a rather large prairie fragment. They state (p. 27) that

> Except for fires, grazing or other disturbance much of this subclimax grassland would undoubtedly pass through a scrub stage in succession and culminate in forest while still other extensive areas would probably remain covered with chaparral.

But in the summary they state that

> The high saturation deficit and low soil moisture content (often reaching the non-available point) of the prairie sites in eastern Nebraska constitute barriers over which the forest trees can scarcely pass. We probably have herein the most ready explanation as to why our natural Nebraska woodlands are confined to the moist slopes of rather narrow valleys, and also the most probable answer to the oft-repeated question as to the treelessness of the prairies in general [p. 47].

The factors given for the maintenance of prairie areas are: *A*, Climatic: (1) temperature, by increasing evaporation; (2) wind, by increasing evaporation and by mechanical effect on tissues; (3) light (insulation) causing greater evaporation. *B*, Edaphic: (4) topography, steepness of the slope causing instability of the soil; (5) low water content. *C*, Biotic and accidental: (6) fire; (7) grazing and trampling; (8) cutting.

It does not appear that fire, grazing, trampling, or cutting is an important factor in the maintenance of these small prairie inclusions, because on account of their isolation within the deep forest these factors as a rule do not affect them. The steepness of the slope would cause some washing of fruits and seeds and young plants; but in depressions and among the prairie shrubs and grass bunches there would be adequate protection from washing. Seedling trees and shrubs occur, as reported by Pool, Weaver, and Jean and by the writer, in spite of the washing.

The data presented in this paper and in the paper by Pool, Weaver, and Jean appear to prove that the dryness of the air, caused by the direct exposure to sunlight and wind, and the low water content of the soil, caused by the high evaporating power of the air and the run-off due to the steep slope, are the determining factors in the maintenance of the prairie inclusions against forest invasion. The air near the surface of the prairie is usually two to four times as dry as in the surrounding shrubs and woods. The water content of the first foot of soil frequently falls below the non-available point in the prairie, while this rarely happens in the shrub and forest. In the prairie, conditions for the germination of tree and shrub seeds are usually not favorable. In case the seeds do germinate, the seedlings are frequently exposed to very dry air and to non-available soil water. The sickly appearance of the tree seedlings observed in the prairie may be accounted for in this way.

Summary

1. Prairie inclusions have been reported as occurring in the deciduous forest climax in Ohio, Illinois, Iowa, and eastern Nebraska.

2. This paper presents the results of an intensive study of a small prairie inclusion near Peru, in southeastern Nebraska.

3. The evaporating power of the air was measured by Livingston standardized atmometers. The daily average losses from May 10 to September 7 were: high prairie (near summit of steep slope) 20.6 cc., low prairie (near base of steep slope) 16.9 cc., shrub zone at base of prairie slope 9.0 cc., red oak-hickory forest below the shrub zone 7.9 cc., bur-oak forest near summit of north slope 9.5 cc.

4. The average of eight total soil-water content readings, at 0–10 cm. depth, from May 18 to July 29 were: high prairie 14.5 percent (wilting coefficient 13.0 percent), low prairie 15.5 percent, shrubs 23.8 percent, red oak-hickory forest 31.2 percent (wilting coefficient 15.5 percent), bur-oak forest 20.8 percent (wilting coefficient 15.2 percent). For the depth of 10–30 cm. the averages were: high prairie 15.2 percent (wilting coefficient 11.9 percent), low prairie 17.0 percent, shrubs 23.6 percent, red oak-hickory forest 27.8 percent (wilting coefficient 12.5 percent), bur-oak forest 20.7 percent (wilting coefficient 12.5 percent).

5. The data presented in this paper tend to prove that the chief factors in the maintenance of the prairie inclusions from invasion by the surrounding shrubs and trees are the great evaporating power of the air caused by

exposure to sunlight and prevailing winds, and the low soil-water content, often falling below the available point.

Department of Biology,
University of Arizona

BIBLIOGRAPHY

1. **Meyer, A. H., et al.** Soil survey of Nemaha County, Nebraska. U. S. Dept. Agr., Bur. Soils. Pp. 38. 1916.
2. **Pammel, L. H., MacDonald, G. B., and Clark, H. B.** The native and cultivated forest trees and shrubs of the Missouri River basin. Proc. Ia. Acad. Sci. **22** : 23–56. 1915.
3. **Pool, R. J., Weaver, J. E., and Jean, F. C.** Further studies in the ecotone between prairie and woodland. Univ. Nebr. Stud. **18** : 1–47. 1918.
4. **Pound, R., and Clements, F. E.** The phytogeography of Nebraska. Lincoln, Nebraska, 1900.
5. **Shimek, B.** Geology of Harrison and Monona Counties. Botanical Report Ia. Geol. Surv. **20** : 426–485. 1911.
6. ——. The prairies. Bull. State Univ. Ia. **6**^{2} : 169–240. 1911.
7. ——. The plant geography of the Lake Okoboji region. Bull. State Univ. Ia. **7** ^{2}: 3–69. 1915.
8. **Vestal, A. G.** Local inclusions of prairie within forest. Trans. Ill. Acad. Sci. **11** : 122–126. 1918.

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1922

President: HENRY C. COWLES
University of Chicago

Vice-President: MARGARET C. FERGUSON
Wellesley College

Secretary: I. F. LEWIS
University of Virginia, University, Va.

Treasurer: IRVING W. BAILEY
Bussey Institution, Harvard University

Councilors:

N. L. BRITTON
New York Botanical Garden

J. C. ARTHUR
Purdue University

C. E. ALLEN
University of Wisconsin

Any one actively interested in botanical work is eligible for membership. Candidates for membership must be recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including one subscription to the AMERICAN JOURNAL OF BOTANY. Candidates proposed for membership, and approved by the Council, may receive one copy of the JOURNAL at $4.00 per year pending their election at the winter meeting of the Society.

Don't Think We Build But One Kind of Greenhouse

Tiffany sells more than one kind and priced scarf pin. They start in at $5.

That $5 one is just as much a Tiffany pin as the $500 one.

It has the same reliableness.

It is Tiffany through and through.

We build just plain, practical purpose houses, void of all embellishments.

Then we build at least three more, each one to meet a particular need and pocketbook.

There are four ranges of costs.

Still every one is built in the same thorough Hitchings way.

They are Hitchings Standard through and through.

Think of us as high-class builders but not necessarily as high-priced ones.

Tell us your need and we will fit a Hitchings house to it.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

PHILADELPHIA
133 So. 12th St.

Vol. IX. JULY, 1922 No. 7

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

CONTENTS

A preliminary study of Claviceps purpurea in culture . . . . Walter W. Bonns 339
Studies in the genus Gymnosporangium IV. Distribution of the mycelium and the subcuticular origin of the telium in G. clavipes . . . . B. O. Dodge 354
Certain relations between root development and tillering in wheat: significance in the production of high-protein wheat . . . . W. F. Gericke 366
The pollination of Marcgravia: a classical case of ornithophily? . . . . . Irving W. Bailey 370
Environmental temperatures of fungi in nature . . . . Neil E. Stevens 385
The reduction divisions in the pollen mother cells of Oenothera franciscana . Ralph E. Cleland 391

Smithsonian Institution
AUG 21 1922
National Museum

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
At Prince and Lemon Sts., Lancaster, Pa.
LONDON AGENTS
WHELDON AND WESLEY, Ltd.
28 Essex Street, Strand, London, W. C. 2

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN
JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
The Thompson Institute For Plant Research, Inc.

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

H. H. BARTLETT,
University of Michigan

ARTHUR H. GRAVES,
Brooklyn Botanic Garden

I. F. LEWIS,
University of Virginia

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Postage to Canada, 20 cents; to other foreign countries (except Mexico and Cuba) 40 cents a volume on annual subscriptions. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume, post free.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or The New Era Printing Co., Inc., Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. IX JULY, 1922 NO. 7

A PRELIMINARY STUDY OF *CLAVICEPS PURPUREA* IN CULTURE

WALTER W. BONNS

(Received for publication October 21, 1921)

INTRODUCTION

The work with *Claviceps purpurea* Tul. herein considered had in its inception a purely physiological objective resulting from the pressure of economic conditions. It is doubtful if the majority of botanists or laymen have a proper conception of the positive economic value of this fungus. To emphasize this point it will suffice to say that the annual import of ergot into the United States for the six years ending with 1919 has ranged from 58 to 112 tons, with a valuation ranging as high as $208,000. Practically all of this, so far as we are aware, is used in the manufacture of pharmaceutical preparations of great importance in obstetrics.

The great commercial sources of the sclerotia in the past have been Spain and Russia. During the recent war American drug manufacturers experienced a great shortage of ergot, and as a result quotations rose by leaps and bounds. In the early part of 1914, high-grade Russian ergot could be bought for 43 cents a pound, duty paid. During the first part of 1920, quotations on this drug ranged between $5.00 and $6.00 a pound.

Our objective, therefore, in undertaking this study was the growth of the fungus on artificial media to determine if under such conditions the physiological principles extracted from the natural sclerotium could be obtained.

LITERATURE

The literature dealing with the physiology of Claviceps is scant. A fairly complete monograph of the genus by Atanasoff (1) gives but three citations of studies of the fungus in culture. According to this writer, Brefeld (7) is credited with being the first to study *C. purpurea* under artificial conditions. The latter grew his cultures from ascospores germinated on bread soaked in nutrient solution. A brief description of the conidial stage is given. Neither sclerotial formation nor anything apparently homologous thereto was obtained in cultures.

About a dozen years after Brefeld's study, Meyer (12) repeated the experiment, starting his cultures, however, from the "honey-dew stage" on infected rye. This paper gives the most detailed description of any

[The **Journal** for June (9: 277–338) was issued July 12, 1922].

so far found, and, because of its bearing on the present work, will be more fully considered later.

Engelke (9), working both with nutrient solution and nutrient agar, furnishes very little detailed information regarding the morphology of the fungus. Brefeld's observations were in general confirmed. Both solid media and nutrient solutions were employed, the former consisting of glucose, ammonium nitrate, mono-basic potassium phosphate, and magnesium sulphate. To this 2 percent agar was added for solid media. The formation of "microsclerotia" on solid media with lowered temperature is noted, but no description or illustration gives any clew as to their nature. This paper is of interest because of the proposal for growing the fungus in culture for the purpose of obtaining a growth possessing the physiological activities of the natural sclerotium. So far we have found no later contribution on this point.

Engelke observed no honey dew in artificial cultures, and regarded the excretion as an increase of stigmatic fluid due to parasitic stimulation and not an excretory product of the sphacelial stage.

Brown and Ranck (8), in a study of forage poisoning, inoculated bean pods, peptone agar, and Paspalum heads with portions of *Claviceps Paspali* sclerotia, securing pure cultures upon the bean medium. The fungus penetrated the pods, but no reproductive bodies were noted. The germination of this species of Claviceps and the production of the sphacelial stage as described by these authors are closely analogous to the conditions in *C. purpurea* as noted by the workers previously cited.

Meyer was guided in the preparation of his nutrient solutions by the chemical composition of the immature rye seed. His liquid medium contained starch, glucose, albumin-peptone, asparagin, di-potassium phosphate, magnesium sulphate, potassium chloride, and ammonium sulphate in proportions to make the ash constituents of the combination approximate those of the rye seed as determined by the organic analysis of Nowacki. The fungus was studied in such solution cultures, or the liquid was added to bread or to cotton. Meyer's descriptions and illustrations of the fungus in culture conform in all essentials with those of Brefeld, but more details are given in the later work. Emphasis is laid upon the rapid growth and prolific spore production, with early germination after abscission. The development of cross walls in the spore before maturity, the presence of two bipolar oil drops before abscission, and the grouping of spores about terminal hyphae or branches after abscission are all noted.

Meyer observed two distinct hyphal stages; in one, the cells were well filled with strongly granulated, fat-containing protoplasm; in the other, the cells were for the most part poor in plasma content, with numerous hyaline vacuolate areas. The latter condition he correlates with advanced hyphal age.

Cultures resulting from inoculation with spores of the honey-dew stage showed spore development in three weeks. A thick mycelial pellicle was obtained on nutrient solutions in 2½ months. Aerial mycelium was produced on bread, potato, and other organic media with inocula from previous cultures. Hyphae on solid media developed spores in scantier amount than those in water cultures. The great viability of the conidiospores was commented upon, and Meyer raises the question whether such spores may not winter over. No heliotropism was noted at any stage of growth.

The plications on solid media noted by Brefeld were confirmed, together with the successive change in pellicle color from yellow to brown, but Meyer does not record the deep violet or purple stage which Brefeld emphasizes. A resting period of the fungus was indicated by a complete cessation of vegetative activity in mid-December of cultures started in July. Renewed activity with continued increase of mycelial growth was observed after a two months' rest period. Cultures in the resting condition gave off, when opened, an odor of trimethylamine.

An attempt was made to distinguish a morphological differentiation between growths on solid and on liquid media. In the former the mycelium was characterized by compact parallel grouping of the hyphae in bundles, whereas in liquid cultures the mycelium branched profusely and grew in all directions.

With respect to sclerotial formation or a tendency thereto, Meyer sustains Brefeld in finding no indications in this direction, even in cultures held for a year. The hyphal elements of the resting pellicle were not pseudo-parenchymatic, as are the cells in a cross section of a sclerotium, but were grouped as masses of short cells, rich in fat. On the older convoluted pellicles a closely compacted parallel hyphal development was noted. From these strands, basidiospore-like elements with terminal spores developed at right angles. The resting stage is regarded as purely conidial or basidial.

EXPERIMENTAL

It was impossible to begin the study here reported with ascospores, as work was started in June, when viable sclerotia of the preceding year's formation were not available. The attempt was therefore made to secure a growth from sclerotia just forming on that season's rye crop. A few rye heads with fairly well developed but still immature sclerotia were obtained in Northern Indiana in June, 1919. These were readily cut transversely with a sterilized razor, the tissue being firm but not hard. Discarding the exposed tissue, portions were removed under sterile conditions from the interior of the sclerotium and transferred to agar.

The medium used in the preliminary work was 2 percent agar (showing a reaction of + 15 Fuller's scale) with decoctions made from rye seed, rye screenings, and germinated rye. The tissue thus obtained was transferred to Petri dishes. Transplants which showed no contamination at the end of a week were transferred to tubes on rye-screenings agar, standing at room temperature.

Sixteen days after transfer to test tubes a very slight growth was indicated, and three weeks later this was distinct in character and identical in all tubes. It had neither bacterial nor gross mycelial characters, resembling in general appearance some Actinomyces in culture. Under low magnification (20 diameters) it showed a smooth, glistening, greyish-white, gelatinous-like surface, peculiarly vermiculated. Repeated transfer to fresh tubes resulted in all cases in growth of similar nature, sometimes progressing directly from the inoculum, and at times first appearing on the surface of the slant at some distance from the point of inoculation as small pustules scattered over the surface of the slant, finally coalescing to form an area similar to the parent growth.

After several transfers the work was interrupted, and, pending resumption of the study, the tubes were sealed in September, 1919, and kept at room temperature. It may here be noted, bearing in mind Meyer's observations of the longevity of conidiospores, that these cultures retained their viability for over a year. This is also in line with Stäger's (14) findings that the conidiospores are viable after wintering over a period of ten months.

No gross mycelial characters had developed in any tubes up to this time, nor did any appear in a period of more than eight months. The consistent nature of successive transplants warranted the assumption of a pure culture, but no detailed microscopic study had been possible. Whether a pure culture of Claviceps, originating from sclerotial tissue, had been obtained, or merely a fortuitous contaminating organism of a parasite on the sclerotium, remained to be established.[1] In January, 1920, smears from several tubes showed a dense stroma, at the periphery of which short hyphal elements and an occasional conidiospore were evident, but no further work towards identification of the fungus was then possible.

In April, 1920, work was resumed by inoculating Petri dishes and tubes of the three kinds of agar with the preceding September cultures. Typical growths appeared on the third day following inoculation, both in tubes and in dishes.

The primary growth preceding mycelium formation was the same as that noted the preceding summer. Smears showed this to be an almost solid field of oval spores in resting condition and in all stages of germination (Pl. XVII, fig. 2). Occasionally a hyphal thread could be noted, and in the majority of these the protoplasmic contents were distinctly segmented, with rounded ends and vacuolate areas between.

Five days from date of inoculation mycelial development began and progressed slowly until checked by the drying down of the media (Pl. XVII, fig. 4). No conidia formation and no sterigmata were noted in the dishes. On the agar slants spores were very numerous.

---

[1] Field inoculations on rye with growths on agar were made in 1920, with apparently positive results. The evidence, however, was rendered circumstantial by loss of identification tags in a severe windstorm.

The cycle from germination to spore formation in the first stage of activity appears to be surprisingly short. Simple fission appears to be a not uncommon mode of multiplication, and spore formation by repeated budding or by the development of a short tube, which then appears to be abscised as one or more spores, is common. Occasionally a spore develops into a promycelium of 150–250 μ with very short side branches of 4–10 μ, or less, the latter developing terminal conidia. The spores, generally ellipsoidal in form, show great variations in size and outline (Pl. XVII, fig. 3), ranging from 2.3 to 10.6 μ in length and from 1.2 to 4.2 μ in width. Such variations may perhaps be ascribed to different stages with respect to germination. Germination from the two poles is quite common (Pl. XVII, fig. 1), a fact noted by Kuehn (11). Spores are frequently septate before germination, and sometimes the wall appears after the formation of a promycelium. The bipolar granular areas noted by Brown and Ranck in spores of *C. Paspali*, and which Meyer in his study regards as oil drops in *C. purpurea*, are conspicuous (Pl. XVII, figs. 2, 7, 9).

Following the mycelial development just noted, fresh inoculations from the same source were made on the same agar media in small 50-cc. Erlenmeyer flasks and on plugs of carrot, potato, and turnip. In addition, 100-cc. Erlenmeyer flasks containing mashes of white corn meal, ground rye seed, and ground rye screenings were used in this series. The inocula were taken from the area of vermicular growth. The new growths upon agar in the flasks repeated the vermicular character until they had attained an average size of 2 to 3 cm. in diameter (Pl. XVI, figs. 1, 2). Following this stage, the surfaces of the media were gradually covered by a very thin, silvery mycelium. No aërial mycelium developed in any agar cultures for several weeks following inoculation. As growth progressed, the central areas in some agar flasks formed a pellicle, becoming raised and slightly folded and convoluted but not in a manner to form the intricate vermicular type of growth (Pl. XVI, fig. 3). The mat developed a thickness of somewhat less than a millimeter. Studies of the central and peripheral portions showed a great predominance of spores in the former and a great mycelial development in the latter.

No growth was obtained on turnip, and a very moderate vermicular growth developing sparse mycelium resulted on potato. On carrot plug growth was rapid and abundant. Within 24 hours following inoculation with a gelatinous portion of the inoculum, this had spread over three fourths of the carrot slant, and within a very few days this growth resulted in a very dense white mycelial cushion which rapidly penetrated the tissue of the plug. This mycelium conformed with descriptions of Brefeld and of Meyer, showing typical hyphae and spore formation with great numbers of abscised spores arranged in parallel fashion along the threads. A similar grouping was commonly noted in growths on agar (Pl. XVII, figs. 5, 7).

Of the three growths on the mashes, that obtained on the rye screenings was an extremely sparse aërial mycelium. On the rye-seed mash a growth of the usual gelatinous-vermiculate type started, which covered the surface of the media in about a week or ten days. From this stage resulted a very dense mat of aërial mycelium, which in the course of several weeks showed a slight tendency to assume a pinkish or purplish hue. The growth upon white corn meal was so rapid and so similar in character to the surface of the corn meal after sterilization that it was difficult to note its progress until it had reached the side of the flask the second or third day after inoculation. Three weeks from the time of inoculation the entire surface and a very large portion of the medium bounded by the sides of the flask were covered with a dense mycelial growth which became strongly convoluted and took on a deep purple-black color similar to that of the sclerotia of Claviceps. As time progressed, the invasion of the fungus continued, although somewhat checked by the gradual drying of the medium. The aërial mycelium in the agar flasks, on the other hand, did not develop uniformly on all three kinds of media, or over the entire surface in any one, but appeared as isolated areas or as a ring.

On agar in Petri dishes, where the growth was confined to the surface and no pellicle of any thickness developed, there was observed the parallel massing of hyphae in strands of from two to five or six threads, which Meyer describes as a morphological variation of the organism on solid media.

We have been unable to confirm this view. No morphological relation of the fungus to solid media of any kind has been observed. On the several kinds of agar other than that in the Petri dishes, and on the other organic solid media with abundant water content, the mycelium developed the usual branching form regarded by previous workers as normal. We incline to the view that the growth variations noted may possibly be a reaction to a surface-tension factor associated with low water content of the medium.

Strands of parallel hyphae were occasionally observed in the same field with branching mycelium. There also appeared to be two types of hyphal development, the threads of one type being two to three times broader than the smaller, predominating kind. The broader filaments were in some instances split at the tips to form long sterigmata, but no spores were observed in such cases.

Our observations also conflict with Meyer's views regarding the greater age of the vacuolated mycelium. In our cultures the latter was most frequently found in the areas of earliest growth, namely in the vicinity of the inoculum. The hyphae of later development, especially the aërial mycelia, have not, even when of considerable age, shown a marked vacuolation or massing of protoplasmic contents as a general characteristic. There appears to be a marked relation between the early gelatinous-vermiculate stage of growth and a lack of normal mycelial development. In this stage, our studies show an extremely short cycle from one spore generation to another, as previously noted. It is in this area of prolific spore production that the short, partially vacuolate hyphae have been most generally observed (Pl. XVII, fig. 1, *A*, *C*; fig. 6). The protoplasmic contents are generally segmented areas of the size and form of spores in early stages. In addition, mycelium in early stages of development has frequently been noted in which non-terminal cells were swelling and assuming the spore form (Pl. XVII, figs. 7, 8, 9). We incline to the view that the phenomena above noted may be regarded as responses by the fungus to cultural conditions and that these growth abnormalities indicate the probability of chlamydospore formation.

The final series of cultures in this preliminary study consisted of 500-cc. flasks containing mashes of white corn meal, yellow corn meal, white potato, sweet potato, carrot, rye seed, rye screenings, string bean, and rye heads after flowering (Pl. XVIII). The latter medium consisted of entire rye heads collected shortly after the flowering period, dried, and finely ground. The weight of solid material used in each case varied from 100 grams in the case of the corn meals to 300 grams for green string beans, with a corresponding variation in the amount of water added in each case, so that the respective substrates after autoclaving resulted in compact solid masses with a water content sufficiently great to overcome the desiccation factor for an extended period of growth. The flasks were inoculated in mid-September, 1920, with inocula from the vermicular area of one of the preceding agar cultures in flasks.

Response to different media was indicated by marked variation in both rate and character of growth. That on sweet potato was the most rapid in the initial stage, the entire surface of the medium being covered with very vigorous aërial mycelium in about one week. At the same time, growths were moderate on the two types of corn meal and on rye seed; string bean and white potato showed slight growth of the commonly observed gelatinous character. Carrot, contrary to the results of our previous work, showed remarkably slow growth. The flask of rye heads showed only a trace. This latter material was not in a compact form, due to its agitation as the result of autoclaving, but we succeeded at this time in shaking the material up and spreading the inoculum, so that better growth resulted thenceforward. The rate of growth after the first week did not maintain the same relative pace, and three months after inoculation the most abundant growth was again to be seen on the two corn meals. In these media the marked purple color and heaping up of the mat in vermiculate form somewhat resembling sclerotia were very abundant (Pl. XVIII, figs. 1, 2). This stage was, of course, preceded, as in the former cultures on corn meal, by that of the dense white aërial mycelium. Next in order of luxuriance was the growth on rye-seed mash (Pl. XVIII, fig. 3). Here the convolutions of the matted surface were much larger, and there was only a hint of a tendency to purple coloration. The aërial mycelium progressed much farther down the sides of the flask than on corn meal. Sweet potato (Pl. XVIII, fig. 9) apparently suffered an inhibition of mycelium production of the aërial type, and the surface was now well convoluted, of a muddy putty color, on the surface of a considerable portion of which was a growth of very short aërial mycelium. On white potato (Pl. XVIII, fig. 6) there developed a very limited light brown, entirely vermiculate growth over the top surface, very small in scale compared to previous growths. String bean (Pl. XVIII, fig. 8) developed the vermiculate type over the entire surface, the convolutions small in scale, densely compacted, with a tendency to develop aërial mycelium, on the surface of the medium contiguous to the sides of the flask. Rye screenings (Pl. XVIII, fig. 4) produced a vermicular growth markedly raised in the center. Mycelium started on the periphery and grew down the side of the flask. The carrot culture (Pl. XVIII, fig. 7) developed no mycelium at the time indicated, was very finely vermiculate at the periphery, and had only partially covered the top surface. The central growth was pustular in character. Growth on rye heads after flowering (Pl. XVIII, fig. 5) was chiefly aërial mycelium and appeared to be working along the surface rather than permeating the mass.

From this time on growth was either extremely slow, or had apparently ceased. Six months after inoculation growth was characterized as follows:

On the corn meals the purple, heavily plicated mat developed over the entire upper surface and over portions of the sides. The earliest stage —the gelatinous—advanced along the sides well towards the bottom. Intermediate between these two was an area of aërial mycelium. The latter had also developed from the convoluted purple upper surface. There was no marked difference between growths on yellow or on white meal. On rye seed the gelatinous, coarsely plicated pellicle covered all surfaces except the base. Aërial mycelium was absent. On rye screenings a finely vermicular pellicle of light-brown color covered the upper surface with no increase of mycelial growth. No further growth was apparent on the rye-heads substrate, which was fairly dry. On white potato the vermicular growth covered the upper surface and a portion of the sides. The carrot flask showed a gelatinous, finely vermicular pellicle covering the top and sides of the substrate to its base. A very sparse, short aërial mycelium was developing in the center of the top. On the string-bean substrate a most compact, finely vermicular growth covered the top and three fourths of the sides. Aërial mycelium had started on the sides only. The flask of sweet potato had previously been broken.

The tendency of the fungus to grow upon the surface of the medium rather than to penetrate the mass to any marked degree—a fact noted by Meyer—was observed in the first series of corn-meal and rye-seed mashes studied. Most probably failure to penetrate is closely related to time and desiccation factors, since the fungus, after the initial stages, grows comparatively slowly. That this appears to be true is indicated in the 500-cc. flask of white corn meal above mentioned, from which the mass was removed *in toto* by breaking the flask, six months after inoculation (Pl. XIX, fig. 1). The corn-meal mash still contained an abundance of moisture, doubtless sufficient for indefinite growth of the fungus. Progression of the pellicle along the surfaces bounded by the flask is readily noted, and the longitudinal section through the center of the mass shows an almost solid layer of fairly compact fungous tissue 2–3 cm. in thickness (Pl. XIX, fig. 2). This is not an accretion of surface growth, since originally the surface of the medium was approximately that of the upper surface of the fungus. It is clearly a growth which has penetrated and apparently completely utilized the substrate in its metabolism.

Sections of this growth at two stages show a type of development completely at variance with Meyer's descriptions and illustrations of what we assume was a similar stage. The older or outer layers do show a denser, compacted growth, but not characteristically parallel; neither has the basidial development of Meyer's description been confirmed. Contrary to his findings that the resting hyphae are not pseudo-parenchymatic, this is precisely what the material shows. Indeed, there is a striking analogy between transverse sections of a sclerotium (Pl. XIX, fig. 6) and of the fungus in culture. There is a marked development of the surface hyphae resembling an epidermal formation (Pl. XIX, fig. 3), and an unmistakable pseudo-parenchymatic formation in the general mass (Pl. XIX, figs. 4, 5). The pseudo-parenchymatic appearance of the sclerotium is doubtless due to a mesh of hyphal elements developed under pressure. It differs in essential characters from sections through the culture chiefly in the smaller size of the cell-like areas and in the compacted cells forming the outer tissues of the sclerotium. We believe the conclusion is warranted that the fungus in culture develops a stage analogous to that found in the sclerotium and that the differences in scale of the cell-like structure are probably due to the fact that in the flask the fungus grew freely, while in the sclerotium growth is confined and subjected to pressure.

As previously stated, no attempt has been made to emphasize the mycological side of the present study, especially since our observations—with the exceptions noted—accord in the main with those of former workers in this field. On the other hand, we have found no reports in the literature dealing with the physiological properties of Claviceps cultures. Engelke's paper, so far as can be learned, does not appear to have been followed by work along the line he proposed. The chief interest in the present work has therefore centered in the physiologically active constituents of the organism in culture, compared with those in the sclerotium of natural development.

The Chemistry and Toxicology of Ergot

The chemistry and toxicology of ergot were studied as early as the eighteenth century, but it is only within recent times that the work of Barger (2, 3, 4, 5), Dale, Ewins (10), and others has resulted in accurate knowledge.[2]

The chief active principles so far isolated from ergot are, according to Barger:

(1) Ergotoxine ($C_{35}H_{41}O_6N_5$), an amorphous alkaloid yielding crystalline salts, which in very small doses produces ataxia, dyspnoea, salivation, gastro-intestinal irritation, and gangrene. The latter is caused by constriction of the arterial circulation which this alkaloid effects.

(2) Histamine (ergamine, β-iminazolyl-ethylamine, $C_5H_9N_3$), a powerful uterine stimulant and blood-pressure depressor.

(3) Tyramine (parahydroxyphenylethylamine, $C_8H_{11}ON$), functioning as the powerful blood-pressure-raising principle in ergot extracts.

(4) Acetylcholine ($C_7H_{17}O_3N$), a depressor of blood pressure.

The standard physiological tests of ergot extract for pharmaceutical use are made for the first three of the above named constituents. Detailed methods are described by Pittenger (13). Histamine action is determined by subjecting freshly excised guinea pig's uterus, suspended in Ringer's solution, to the standard ergot dose, which is added to the solution, the resulting muscular contraction being recorded on a kymogram apparatus. For the tyramine test, cats, dogs, or rabbits are used, the drug being injected intravenously and the rise in blood pressure being recorded. Tests for ergotoxine are most generally made upon the comb of the domestic fowl. Injection is intramuscular. The action generally occurs within an hour, evidenced by a very distinct bluing of the comb and sometimes of the wattles. Attendant symptoms are restlessness, drooping of the head and tail, diarrhoea, and inability to stand normally erect.

Since the greatest development, and apparently the most advanced stages of growth, appeared in the cultures on corn meal, they were the ones selected for tests of physiological activity. For the first of these the growth on corn meal in 100-cc. flasks was used. In this instance growth did not permit a ready separation of the fungus from the substrate, and the entire mass was air-dried to constant weight, and then ground and percolated for the fluid extract. Forty-two grams of substrate yielded 10.86 grams dry weight of material for percolation. On the basis of dry weight the total fungus was estimated as not to exceed 2 percent of the total dry weight used in extraction. Test of the extract from this source in standard dose produced no distinctive bluing of the comb of a White Leghorn cock, and only a slight indication of increased blood pressure in a rabbit.

---

[2] An excellent summary, with bibliography, of the entire subject of ergot, embracing its history, botany, medicinal properties, aetiology, chemistry, etc., has been made by Barger (6).

The second test was made with an extract in which the fungous material largely predominated. To attain this end, the cultures were grown on a thin layer of corn-meal mash in Petri dishes (10 g. of meal, 10 cc. of 1 percent peptone solution, and 10 cc. tap water). These cultures, inoculated from one of the 500-cc. flasks in the series previously discussed, grew rapidly, passing through all the previously described stages (Pl. XX, fig. 1) and developing at a comparatively early stage the soluble purple color (presumably sclererythrin, a physiologically inert substance). This diffused quite readily to the lower surface of the substrate (Pl. XX, fig. 2). Sterile water was added when necessary to promote growth. At the end of three months, four dishes were dried to constant weight, and their contents were removed, ground, and extracted by the standard method. The finely ground material had the general appearance and the peculiarly musty odor of ground ergot sclerotia. Forty grams of the original corn meal yielded 22.2 grams of dried powdered fungus and substrate mixture. Microscopic examination showed that this contained less than thirty percent (estimated) of unaltered corn meal.

In color and odor the fluid extracts from this material—as, indeed, in the one previously made, and in the one to follow—were characteristic of standard ergot extract except that in shade of color they were somewhat lighter, resembling more closely a percolate of domestic rather than of foreign ergot.

With this extract tests were made as follows:

For tyramine (*parahydroxyphenylethylamine*). Blood-pressure tests were made on the rabbit and cat. Negative results were obtained on a rabbit with both fungous extract and standard U. S. P. ergot extract. On cats the latter produced increase of blood pressure characteristic of standard ergot preparations. The extract of the fungous culture effected a lowering of pressure but showed no pressure increase.

For histamine. A uterine muscle-contraction test was made on guinea pig. The kymogram chart (Pl. XXI, fig. 1) shows the results obtained. *A* and *C* indicate the contraction resulting from the addition of standard U. S. P. ergot extract, 1–10,000. This represents 1 gram of powdered ergot per 10,000 cc. solution. *B*, *D*, and *E* are the contractions obtained with extracts from the culture in strengths of 1–3300, 1–5000, and 1–7000, respectively. The figures beside each wave are the distances in millimeters from crest to base line.

It is clear that there was present in the extract a principle causing uterine contraction and ranging in strength about from 1/3 to 7/10 that of standard ergot preparations.

For ergotoxine. The tests for this alkaloid on White Leghorn cocks were in all cases negative, so far as indices of comb-bluing are concerned. Two tests were made on a bird previously standardized for his reaction to U. S. P. ergot, the injections being made at intervals of four or five days to permit complete recovery from the previous doses. The dose was in each instance 2½ times the strength necessary to produce a very distinct comb-bluing of the same bird with standard ergot extract. In neither case did the injection of culture extract produce anything more than a very slight bluish tinge of portions of two tips of the comb. The slight color changes may well be ascribed to the general physiological reaction of the fowl to the injection, as the comb is a fairly sensitive index of condition, but no deductions other than negative are warranted with respect to ergotoxine action.

On the other hand, there was clear evidence of toxaemia. One half hour after injection the bird was distinctly sick, exhibiting general excitation, drooping of head and tail, and shaking of comb. Diarrhoea was noted in both trials about one half hour after injection, in one instance being more pronounced than in the other. One hour after injection the bird was very weak on its legs (Pl. XXI, figs. 3, 4), inclined to squat or to lie flat, with sunken head (Pl. XIX, fig. 5). A complete return to normal condition was not effected for several days.

A final trial of extract of pure fungus was made with material taken from abundant growth in the 500-cc. flask of corn meal illustrated in Plate XIX, figures 1 and 2. With the exception of a small portion reserved for microscopic study, the entire upper layer of fungus here shown was removed and extracted after drying. Fifty-six and eight tenths grams of fungus produced 5.8 grams of air-dried material for extraction. The latter had the same gross characters as previous material. A dose seven times the normal produced no effects of any kind in a healthy cock. The extract, when tested chemically, showed no trace of alkaloids.

Controls with extracts of autoclaved and dried corn meal were carried on with the foregoing tests, and in all cases gave negative results.

Discussion

The present study confirms in great part the descriptions by former students of *C. purpurea* in culture, but opposes them in several particulars—notably in regard to the question of morphological variations and the development in cultures of advanced age of a stage distinctly analogous to the pseudo-parenchyma and epidermal layers of the natural sclerotium. The development of the conidial stage directly from the sclerotium without germination and without formation of ascospores has been demonstrated.

The data presented give indications of the presence in Claviceps cultures as here grown of but one of the commonly recognized active principles of ergot—histamine. There is at present no ground, however, for a belief in the presence of ergotoxine in the cultures, in the light of chemical examination. The qualitative test was made upon a very small amount of material, so that analyses upon a larger scale will be necessary before this fact will be established with certainty. It is highly possible, however, that the presence of the alkaloid is directly associated with changes involving sclerotial formation, and the present work conforms with that of its predecessors in failure to obtain this resting stage under cultural conditions.

In the light of the present study it appears extremely doubtful that the artificial culture of Claviceps possesses practical application. There are several factors indicated by this preliminary work which need to be determined before a definite conclusion in this respect may be drawn.

(1) The organism must be grown in quantities sufficient to make practicable a careful quantitative and qualitative study of the physiologically active principles present.

(2) The marked variation in growth response to different media indicates that its behavior and constitution under cultural conditions of known pH values would afford a valuable field of investigation.

The writer gratefully acknowledges the coöperation of Messrs. Edward Swanson and C. H. Hargreaves of the Department of Experimental Medicine of this company, who prepared the extracts and performed the physiological tests reported. Thanks are due Mr. G. Rudolph Miller for instruction and generous assistance in photomicrography, and to Dr. M. W. Gardner of Purdue University for similar favors. Mr. F. J. Bacon, assistant botanist, has been of great service in preparing material for microscopic study and in general assistance.

Botanical Research Laboratory,
Eli Lilly and Company, Indianapolis, Ind.

REFERENCES

1. **Atanasoff, D.** Ergot of grains and grasses. Bur. Pl. Ind. U. S. Dept. Agr. Pp. 127. 1920.
2. **Barger, G., and Carr, F. H.** The alkaloids of ergot. Jour. Chem. Soc. **91**: 337–353. 1907.
3. ——**, and Dale, H. H.** Ergotoxine and some other constituents of ergot. Biochem. Jour. **2**: 240–299. figs. 1–8. 1907.
4. ——. Isolation and synthesis of p-hydroxyphenylethylamine, an active principle of ergot soluble in water. Jour. Chem. Soc. **95**: 1123–1128. 1909.
5. ——**, and Ewins, A. J.** The alkaloids of ergot. Part 2. Jour. Chem. Soc. **97**: 284–292. figs. 1–5. 1910.
6. ——. Ergot and ergotism in the past. Pharmaceut. Jour. **105**: 470–473. 1920. (Also in Nat. Druggist **51**: 82–84, 141–142. 1921.)
7. **Brefeld, O.** Untersuchungen aus dem Gesammtgebiete der Mykologie (Ascomyceten II) **10**: 192, 193. Pl. 5, figs. 63–67. 1891.
8. **Brown, H. B., and Ranck, E. M.** Forage poisoning due to *Claviceps Paspali* on Paspalum. Miss. Agr. Exp. Sta. Tech. Bull. 6. Pp. 35, figs. 1–18. 1915.

9. **Engelke, C.** Neue Beobachtungen über die Vegetationsformen des Mutterkornpilzes *Claviceps purpurea* Tulasne. Hedwigia **41**: 221, 222. 1902.
10. **Ewins, A. J.** Acetylcholine, a new active principle of ergot. Biochem. Journ. **8**: 44–49. 1914.
11. **Kuehn, J.** Über die Entstehung des Mutterkorns. Ber. Sitz. Naturforsch. Ges. Halle 12–14. 1863.
12. **Meyer, B.** Untersuchungen über die Entwickelung einiger parasitischer Pilze bei saprophytischer Ernährung. Landw. Jahrb. **17**: 915–945. Pls. 16–19. 1888.
13. **Pittenger, P. S.** Biochemic drug assay methods. Pp. 158, 88 fig. 1914.
14. **Stäger, R.** Infectionsversuche mit überwinterten Claviceps-conidien. Mycol. Centralbl. **1**: 198–201. 1912.

EXPLANATION OF PLATES

PLATE XVI

Growths of *Claviceps purpurea*

FIG. 1. On rye-screening agar, early stage.

FIG. 2. On rye-screening agar, late stage.

FIG. 3. On rye-seed agar, late stage. Photographed when agar had begun to shrink from sides of flasks. Appearance of growths unaffected.

PLATE XVII

FIG. 1. Germinating spores. *A–C*, × 900; *D*, × 385.

FIG. 2. Spores, rye-screening agar, vermiculate area. × 900.

FIG. 3. Spores, rye-seed agar, vermiculate area, later stage. × 900.

FIG. 4. Mycelium, Petri dish culture, rye-seed agar. Full size.

FIG. 5. Mycelium and spores, rye-screening agar. × 900.

FIG. 6. Mycelium on carrot. × 385.

FIG. 7. Mycelium on rye-screening agar. × 900.

FIG. 8. Mycelium on carrot. × 385.

FIG. 9. Mycelium on rye-screening agar. × 900.

PLATE XVIII

Growths on mashes after three months

FIG. 1. On white corn meal.

FIG. 2. On yellow corn meal.

FIG. 3. On rye seed.

FIG. 4. On rye screenings.

FIG. 5. On rye heads after flowering.

FIG. 6. On potato.

FIG. 7. On carrot.

FIG. 8. On string bean.

FIG. 9. On sweet potato.

PLATE XIX

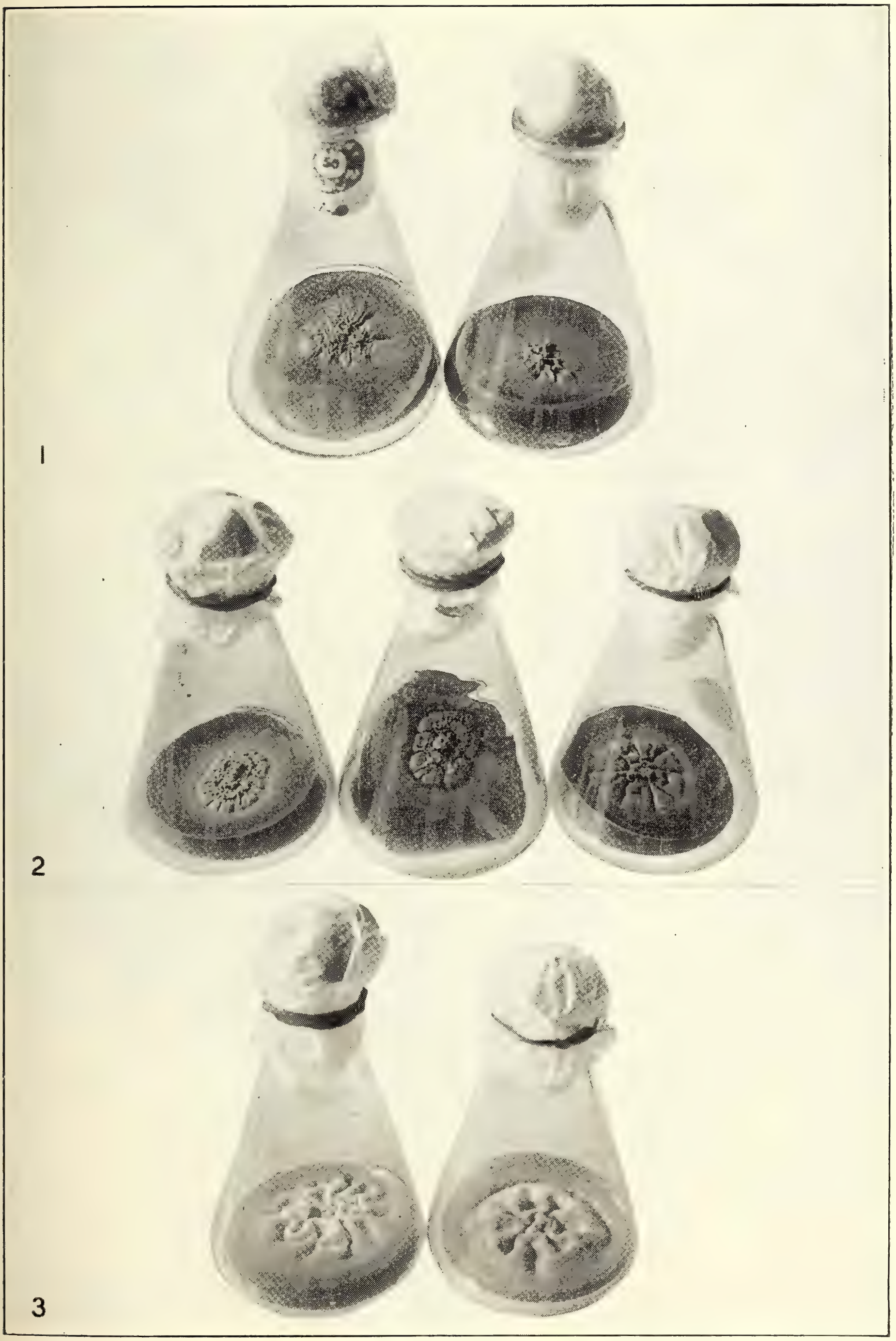
FIG. 1. Six months' growth on white corn meal.

FIG. 2. Longitudinal section through same.

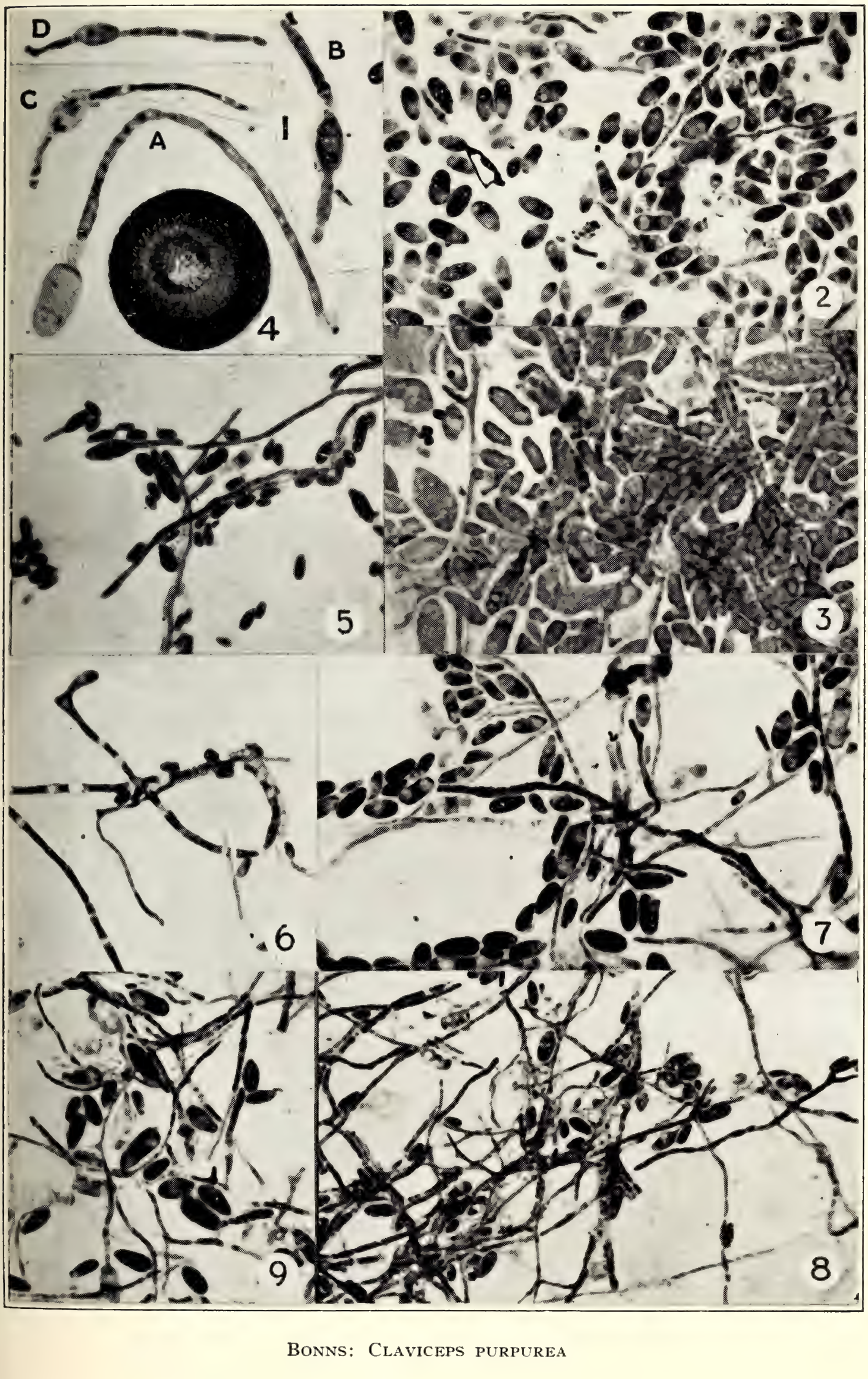
FIG. 3. Section through surface portion of growth in the same flask, showing tendency to epidermal formation. × 200 (approximate).

FIGS. 4, 5. Sections through center of growth, showing pseudo-parenchyma. × 200 (approximate).

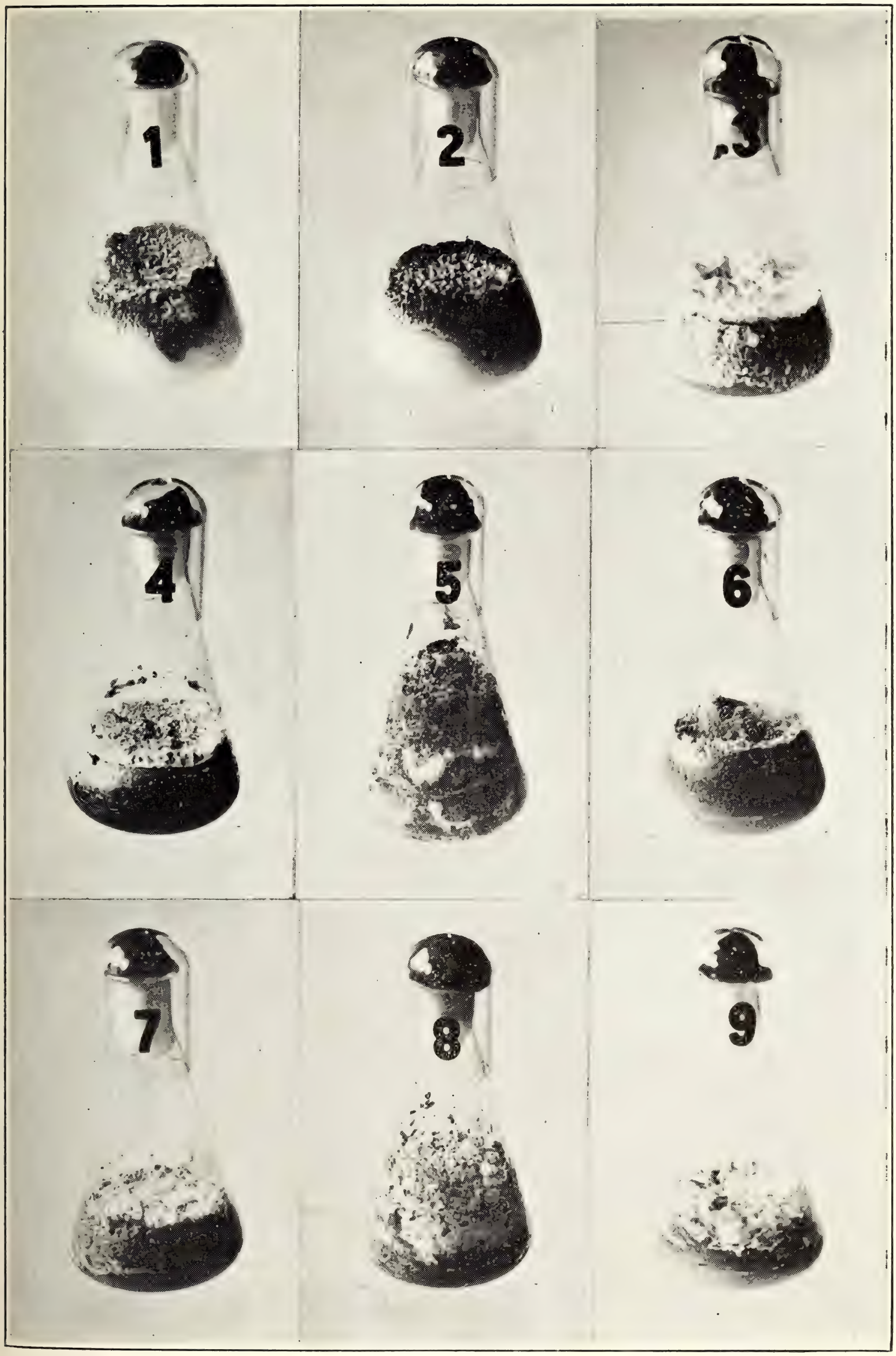
FIG. 6. Section of ergot sclerotium. × 200 (approximate).



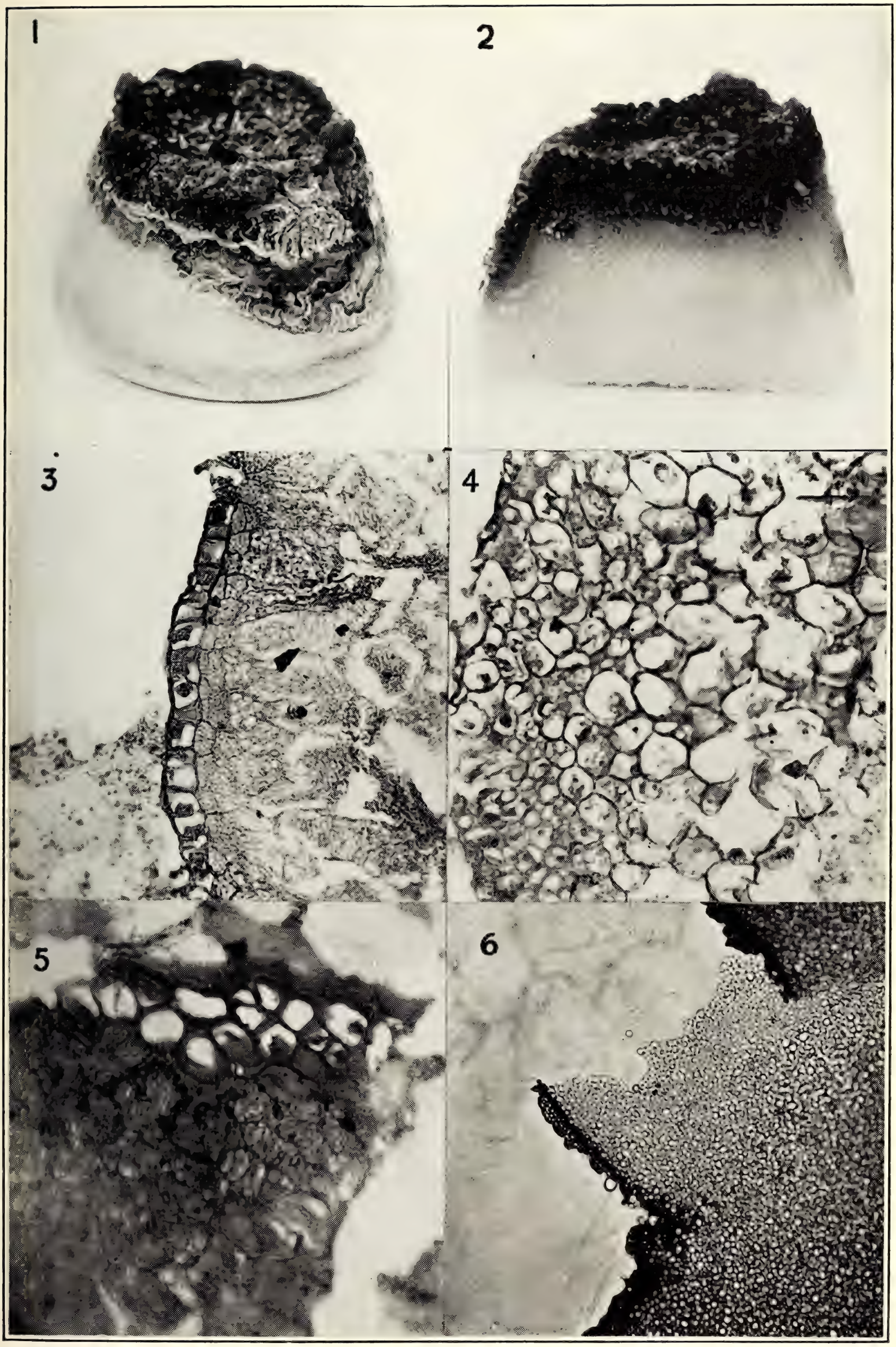
BONNS: CLAVICEPS PURPUREA


D
B
C
1
A
4
2
5
3
6
7
9
8

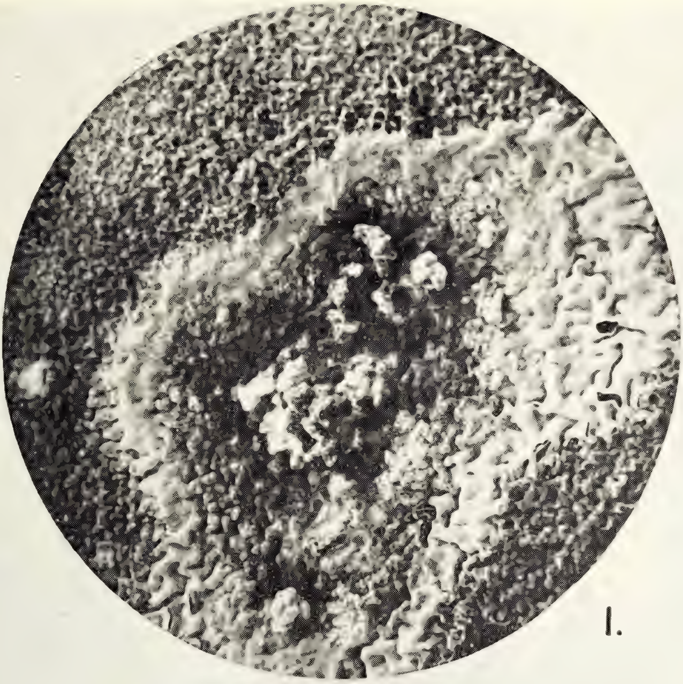
Bonns: Claviceps purpurea



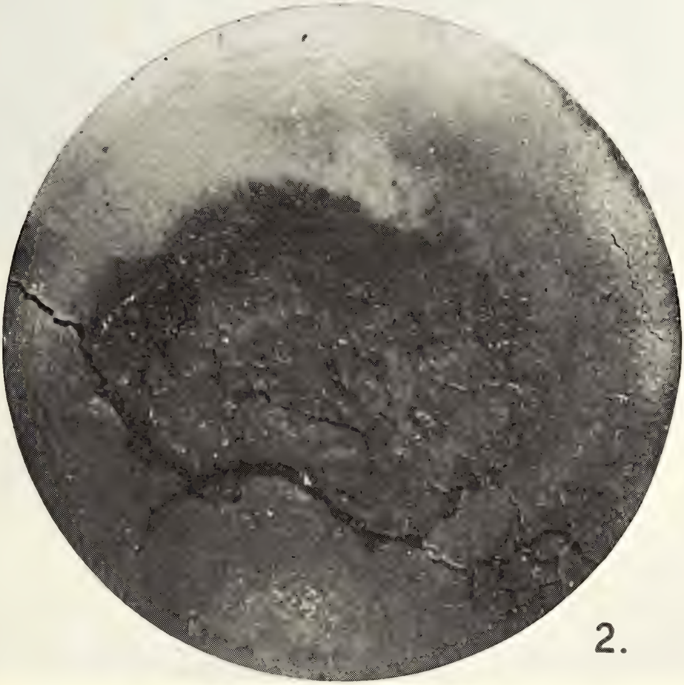
Bonns: Claviceps purpurea



BONNS: CLAVICEPS PURPUREA

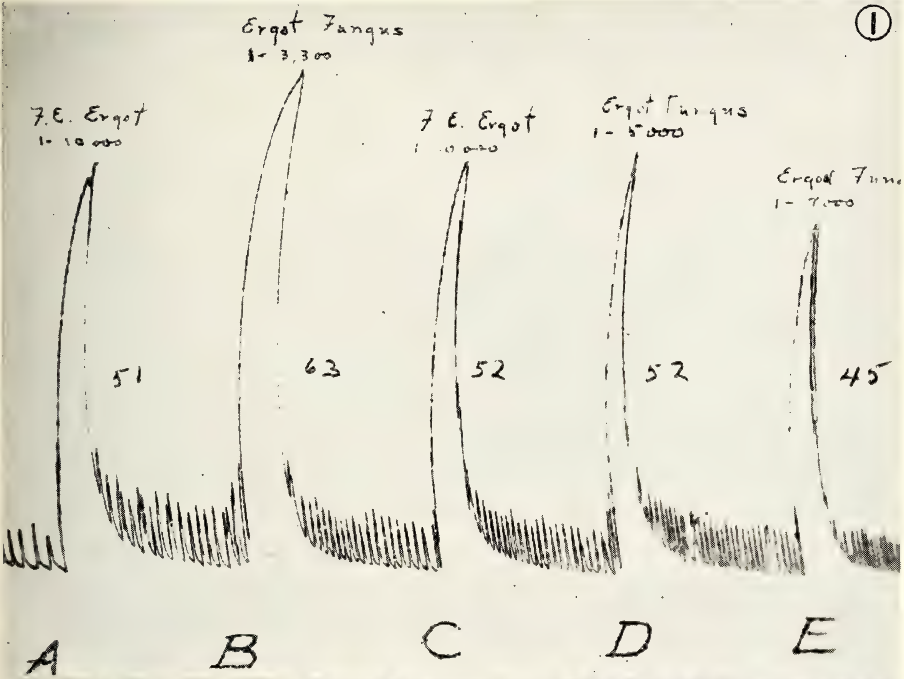


1.



2.

Bonns: Claviceps purpurea


①

2

3

4

5

Bonns: Claviceps purpurea

PLATE XX

Growth on corn meal in Petri dish

FIG. 1. Top.

FIG. 2. Bottom, showing diffusion of pigment through substrate.

PLATE XXI

FIG. 1. Kymogram record, showing contraction effect of standard fluid extract of ergot (*A* and *C*), and of extract from Claviceps cultures (*B*, *D*, and *E*), at different dilutions, on uterine muscle of guinea pig. Figures give height in millimeters of wave from base line to crest. Extract concentrations at crests.

FIG. 2. Normal posture of White Leghorn cock.

FIGS. 3, 4, 5. Effect of Claviceps culture extract on posture of White Leghorn cock.

STUDIES IN THE GENUS GYMNOSPORANGIUM IV. DISTRIBUTION OF THE MYCELIUM AND THE SUBCUTICULAR ORIGIN OF THE TELIUM IN *G. CLAVIPES*

B. O. Dodge

(Received for publication December 5, 1921)

A parasitic fungus in its periods of exploration and attack seeks out those regions where its essential foods are readily available. The host may react to the irritations in such a way as to lead to the formation of abnormal growths which can be relied on in diagnosing the disease. Most species of Gymnosporangium are, aside from their morphological differences, easily distinguished, because each species may differ from the others not only as to the tissues invaded but also as to the manner of the attack.[1] *G. clavipes* Cke. & Pk. on *Juniperus virginiana* differs from all other species studied by the writer in the very limited or superficial way in which the mycelium invades the host, and in the location of the telial sorus as it is formed in leaves or young stems. Hitherto it has been thought that the telia of all Gymnosporangia arise beneath the epidermis. Those of *G. clavipes* are not subepidermal but are developed in the outer wall of the epidermal cells. They might be called *subcuticular*, but, as will be noted later, there is no single word in English that is strictly applicable.

Ducomet[2] made a special study of those spot-disease fungi which live at least a part of the time beneath the cuticle of the host. The method of invasion by such fungi is quite as characteristic as the location of the spore-bearing structures with respect to the cuticle or epidermis. Ducomet found that certain species develop directly beneath the cuticle. These would be called subcuticular in a strict sense. Other species develop in the outer wall of the epidermal cells, in that part which is known as the cuticular layer and which is composed of cellulose and cutin. He proposes such names as *intracuticulaire*, *subintracuticulaire*, etc., terms which do not convey very well the meaning desired.

Arthur[3] has emphasized the taxonomic importance of knowing the location of the sori in rusts.

---

[1] For further discussion of this question see Wörnle, P. Anatomische Untersuchung der durch Gymnosporangium-Arten hervorgerufenen Missbildungen. Forst. Nat. Zeitschr. **3** : 68–84, 128–172. 1894; Dodge, B. O. Studies in the genus Gymnosporangium I. Notes on the distribution of the mycelium and the germination of the aecidiospore. Brooklyn Bot. Gard. Mem. **1**: 128–140. 1918.

[2] Recherches sur le développement de quelques champignons parasites à thalle subcuticulaire. 1907.

[3] Résult. Sci. Congr. Bot. Vienne, p. 333. 1906.

> Im allgemeinen zeigt die Tiefe in den Geweben des Wirtes, den sich der Pilz zum Sitz seines Lagers ausgewählt hat, eine gewisse Beziehung zu der phylogenetischen Entwicklung.

In certain subfamilies pycnia are always subcuticular, other sori may or may not be so situated. In several genera all sori are subepidermal.

Grove[4] also believes that the placement of sori is an indication of the trend of evolution. The transference of the spores to a place either (1) beneath the cuticle, (2) in the epidermal cells, or (3) just beneath the epidermis, and their aggregation into definite sori would be advances in adaptation, "and the most effective of all (the subepidermal sorus) is alone to be met in the highest groups."

The writer[5] investigated the origin of the teliospore in several of our common Gymnosporangia and found that here, unlike the condition in all other known rusts, the teliospores are formed from buds arising out of subterminal cells of the plectenchymatous primordium. After discovering that the telial sorus of *G. clavipes* is subcuticular, it was thought that it might differ in the origin of the teliospore from other Gymnosporangia and thus conform to the mode described for all other rusts. However, as will be shown later, here too the preteliospore degenerates, functioning only as a buffer cell just as it does in the other forms previously described by the writer.

THE HOST–PARASITE RELATIONSHIP

The first leaves of the actively growing shoot of the young red cedar are called subulate in contrast with the scale leaves formed later. The free blade is somewhat less than a centimeter long and the basal portion runs down on the stem about the same distance, varying according to the rate of growth of the stem. As there are three rows of leaves, a cross section of the stem shows the three decurrent leaf-base ridges. The parenchyma of these leaf ridges merges with that of the cortex of the stem, so that there is frequently no definite separating line (Pl. XXII, fig. 3).

The leaf is provided with stomata along its upper (proaxial) surface and on the abaxial side downward from a line about opposite the end of the resin duct, or a little above the line of the leaf axis. There is a single vein in the leaf. A layer, one or two cells thick, of stereome extends along the lower side next the epidermis and continues down the decurrent basal portion, being interrupted wherever stomata are present.

In addition to the true cuticle, the common covering of the leaf and young stem, the epidermal cells are further provided with a layer which can easily be differentiated by proper staining. According to Von Mohl,[6] the cuticular (cuticularized) layer as distinct from the true cuticle is that part of the wall of the cell which has a cellulose matrix impregnated with cutin. Irregular tooth-like projections alternate with notches and pits of a different consistency, so that this region appears more or less porous or granular. The cuticular layer is thickest on the outer wall of the cell, but it extends down between the epidermal cells some distance, and may even occur along the inner wall.

---

[4] The British rust fungi, p. 79. 1913.

[5] Mycologia **10** : 182–193. 1918.

[6] Bot. Zeit., p. 502. 1847.

The epidermal cells of the cedar are oblong or brick-shaped, easily distinguished from the palisade mesophyll on the upper or proaxial side of the leaf. Other features necessary to the ready understanding of the pathological aspects of the subject are brought out by figures in the text and plate.

Infection experiments and field observations made during a number of years indicate that the first telia of *G. clavipes* on *Juniperus virginiana* are formed either directly on the leaves or, more commonly, at the margins of the decurrent leaf bases or in leaf axils of branches not over two or three years old. Later, as cork is laid down, sori break through in the ordinary manner. We shall therefore follow here the course of the fungus during the first year or two of its life in the host.

The Mycelium in the Leaf

Infection takes place on the proaxial side of the young leaf, or directly on the young stem at the base of the leaf. Primary infection can not occur

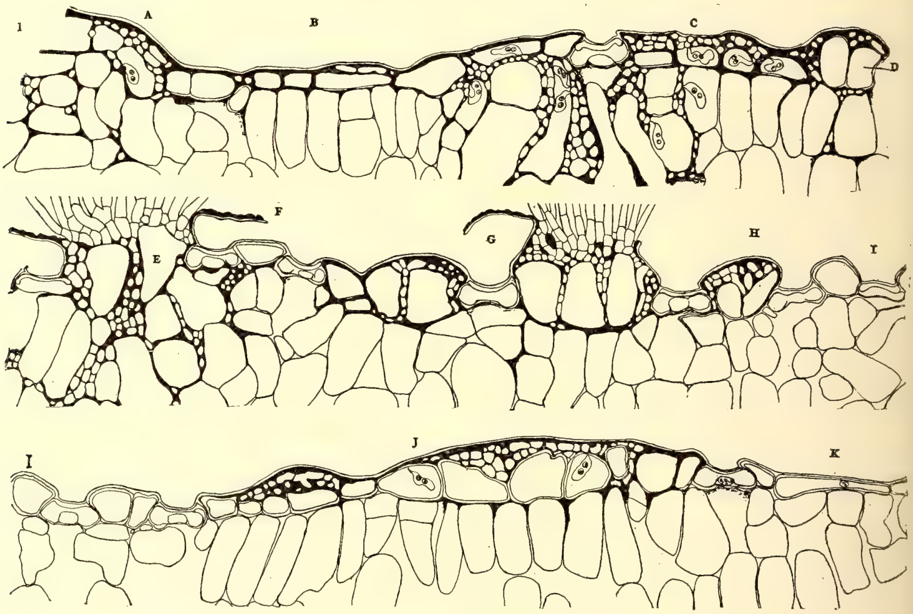


FIG. 1. *A* to *K*, portions of longitudinal section of a leaf of red cedar infected with *Gymnosporangium clavipes;* shows two sori and the manner of advance of the mycelium. Intercellular hyphae have penetrated little beyond the palisade cells, which in many cases have been stimulated to divide. See text for further description.

on stems protected by a layer of cork cells. The mycelium may be confined to the leaf two or three years, developing sori each season; the leaf may then die off without the fungus having been able to reach the stem tissues below. After entering the leaf, the mycelium invades the region between the cuticle and the cellulose walls of the epidermis on the proaxial side. The effect of the fungus on this part of the cell wall is usually marked by considerable swelling, and the disorganized substances take the stains very readily (indicated by shaded portions in my figures). The cell walls of the hyphae also thicken so that it is often difficult to say where these end and the disorganized cuticularized region of the host begins. Haustoria are found in epidermal cells (fig. 1, *C*), sometimes even in the guard cells of stomata. As noted, the fungus explores and feeds in the cuticularized layer, but it may go deeper and invade the palisade mesophyll tissue. None of my sections shows hyphae on the abaxial side of the leaf. Figure 1, *A* to *K*, represents about three fifths of that portion of the leaf which shows the presence of the fungus. Hyphae are found near the epidermis along half the entire length of the leaf. At *A*, palisade cells have divided and the epidermal cells are swollen. At *B*, only a few hyphae are visible but beneath the stoma the mycelium is massed between the palisade cells. The cuticle is raised considerably at *C*, and the cuticularized layer beneath it is much disorganized. At *D*, there is a swollen supporting cell of the stoma which is shown at the left below. Exploration laterally in the cutinized wall is very slow.

At *E* in figure 4 the epidermis is normal. All traces of the fungus in this section are indicated in the figure, and it is evident that no hyphae approach the conducting system *F*. The attack on the leaf is certainly very limited and superficial, but not more so than it is on the young stems or old tree trunks, phases which will now be considered.

Distribution of the Mycelium in Young Stems

Vigorously growing terminal regions of young stems are the most susceptible to infection, either directly or through the invasion of the fungus by way of the leaf axils. At the time of the formation of the first sori, which will be either about ten months or a year and ten months after infection, the stem will still be green and devoid of cork tissue, the greater portion being covered by the decurrent leaf bases. The diagrams *A–F* in figure 2 were made from a series of sections of a small branch of plant no. 403 which had been infected in August, 1915,[7] the first sori appearing in the spring of 1917. The number of sections 7 μ thick between those diagramed is given in the figure. Beginning with diagram *A*, the mycelium represented by shading is shown to occupy the three regions in the cortex between the decurrent leaf bases (*L*), not approaching the cambium; even the phloem tissue is some distance beyond. Passing up the stem about a millimeter, diagram *B* shows that each mycelial strand is being broken up or split as a new set of leaf bases (*L*′) appear. Only a tenth of a millimeter farther (*C*) we find that each strand has been completely split and that there are now six distinct regions in the cortex penetrated by the fungus, a pair of mycelial strands for each leaf base. At *D* hyphae have cut in beneath one leaf vein, and the union of the members of the other two pairs of hyphal strands is completed thirty-three sections above at *E*. Note that there is some mycelium invading the epidermis on the proaxial side of the leaves near the lines of attachment. At the point *F*, a few sections above, two leaf blades are detached, and subcuticular and intercellular hyphae have penetrated still farther into the leaves. The last of these hyphae is seen in the twentieth section (not shown) above *F*, proving that the mycelium penetrated only 210 μ into the leaf above the line of its attachment to the stem. These gaps between strands of mycelium continue up and down stems even in those showing three rings of wood, and one wonders if the fungus would ever occupy the entire cortex, especially as he finds old trunk infections confined to only a small part of the circumference. But in an infection such as the one diagramed in figure 2 the strands actually interlace, weaving in and out around the veins of the leaves and forming a closed network system in the cortical region of the

---

[7] See Bull. Torrey Bot. Club **45** : 287–300. 1918.

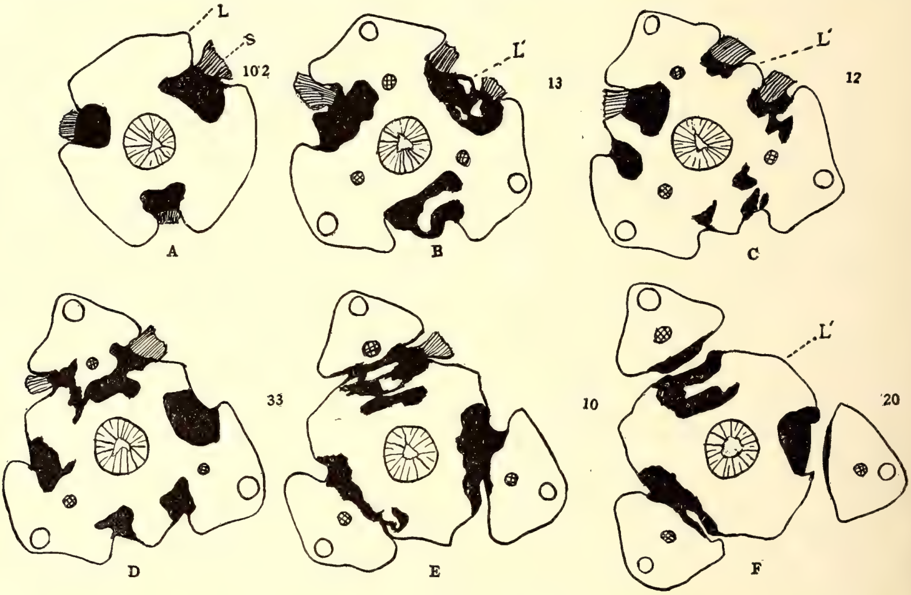


FIG. 2. Diagrams *A* to *F* made from cross sections of a branch infected with *Gymnosporangium clavipes*, Aug., 1915, cut March, 1917. Shaded portions show location of mycelium and sori. A longitudinal diagram would have shown these mycelial strands interlacing, forming a closed network as they weave around the veins leading into the leaves. See text for explanation and discussion.

young stem. After the leaves are shed, the fungus, closing the gaps, will be found in the cortex around the entire circumference of every section. The diagrams bring out very strikingly the fact that the mycelium never gets very near the conducting systems of the host. Wörnle (*l.c.*) states that he found mycelium of *G. clavipes* in the bast. He was apparently mistaken in this regard, as I have seldom if ever found hyphae approaching phloem very closely.

The Mycelium in Trunk Infections

The fungus at first advances longitudinally in the stem one to three centimeters each year but circles it very slowly, sometimes taking several years in the process. In many cases of trunk infection the mycelium is always confined to a small region and never completely girdles the tree. Where the stem is attacked in a rapidly growing region, such as at the end of the main axis or at the tip of a well exposed branch, the fungus is outstripped by the host, the infection being recognizable in later years by the slightly fusiform swelling. If infection takes place on a slowly growing side branch, the parasite grows upward with the host, penetrating new branches, frequently causing, as a consequence, distortions like small witches' brooms.

The main trunk of the cedar may also become infected, even after many layers of cork have been laid down, as a result of the downward growth of hyphae from an infected branch. Such secondary infections may account for the types of injury characterized by black patches a few inches in diameter on trunks of large trees, showing how little damage may be caused by this species during the fifty or one hundred years which have passed since infection occurred.

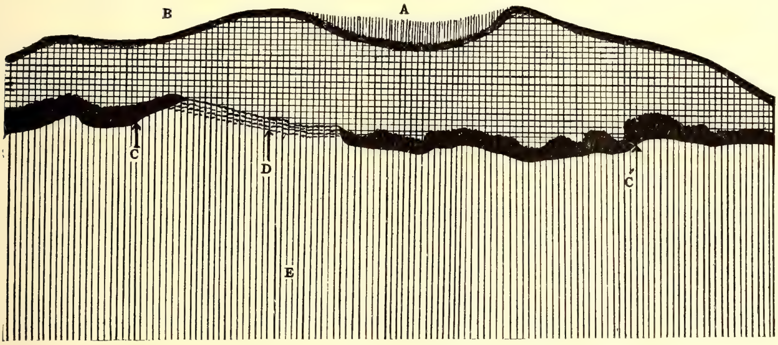


Fig. 3. Diagram representing a small portion of the cortex, Nov. 25, 1917, of an infected cedar limb twenty years old, showing the manner in which the wound callus cuts off the old sorus and adjacent infected tissue of the preceding spring. *A*, remains of teliospore stalks. *B*, wound callus cork. *C*, cork cambium and one or two layers on either side among the cells of which hyphae have invaded from the margins. *D*, uninfected gap of cork cambium which will soon be invaded. *E*, uninfected cortex, which extends much farther down.

A good demonstration of the presence of the fungus in the cedar can be made from free-hand sections. Fixed with Flemming's agent, the invaded tissues are blackened so that one sees a narrow ring consisting of phellogen and one or two adjacent rows of cells just below the cork, perhaps not over 5 to 10 percent of the cortical zone (Pl. XXII, fig. 5, and text fig. 3). Bleached and stained with the triple stain, characteristic binucleated haustoria and hyphae become visible. It is undoubtedly the vigorous attack on the phellogen cells by the fungus that prevents the formation of the normal amount of cork. As soon as the spores have germinated in the spring a new cork cambium is developed in the cortex beneath the sorus, so that as the callus is formed all traces of the fungus disappear as last year's infected tissue is lifted up and sloughed off. This would leave pockets in the cortex beneath each old sorus devoid of fungus were it not for the reëntrance of the parasite from the sides (*C*, *C′* , fig. 3). The diagram represents this condition, Nov. 25, 1917, in a small area from an infected limb twenty years old. Remains of the old telium (*A*) are still visible above the wound callus. There is a small region (*D*) of phellogen still free from the parasite. Now, if this infected area had been examined in April of the preceding spring, the hyphae beneath the sorus would have been confined to the region of the cork cambium as shown in figure 4, Plate XXII. While, as stated, hyphae in such infections usually occupy only the two or three outermost layers of the living cortex (Pl. XXII, fig. 5), yet one often finds the fungus halfway down to the phloem.

The Origin of the Sorus

The telial sorus may be said to originate in that region where the spore buds are formed. The sorus of *G. clavipes* would be subcuticular in a broad sense because the subterminal cells in the plectenchymatous primordium are formed in the cuticularized layer of the host, which would be somewhere between the cuticle and the lumen of the epidermal cell beneath.

The Telium in the Leaf

In view of the very superficial attack on the leaf and young stem tissues by the fungus, which appears to get much of its nourishment from the epidermis, it does not seem strange that the sorus primordium should be found originating in the cuticularized layer. The point at which a sorus will develop is usually marked by a massing of hyphae, so that the cuticle is widely separated from the underlying cells (fig. 4, *D*). As the primordium enlarges and spores are formed, the cuticle, carrying some portion of the cuticularized layer which has become more or less disorganized, breaks away along a line and folds back so that the nature of the sorus can be determined very readily. A small sorus is shown at *E*, figure 1, where the mycelium is deep-seated. The large cells (*E*) at the base of the sorus are the supporting cells of the stomata on either side. Another sorus is shown at *G*. Hyphae pass around the stoma, showing in this section again at *H*, where both supporting epidermal cells are enlarged. The effect of the fungus near by is seen at *I*, hyphae appearing again in the section exploring new regions subcuticularly toward *J*. The epidermal cell at *K* is normal; no hyphae have advanced beyond this point in the leaf.

The nature of the telium is even more evident in the cross section outlined in figure 5, where the epidermal layer is still unbroken from the stoma at the left to the limits of the sorus at the right. The cells of the epidermis are somewhat swollen, but 70 μ farther up some of them are nearly normal (fig. 6). Hyphae have crowded in between the longitudinal rows of epidermal cells at *A*, and two of these have been shoved aside, but they can be seen in the adjacent sections of the slide.

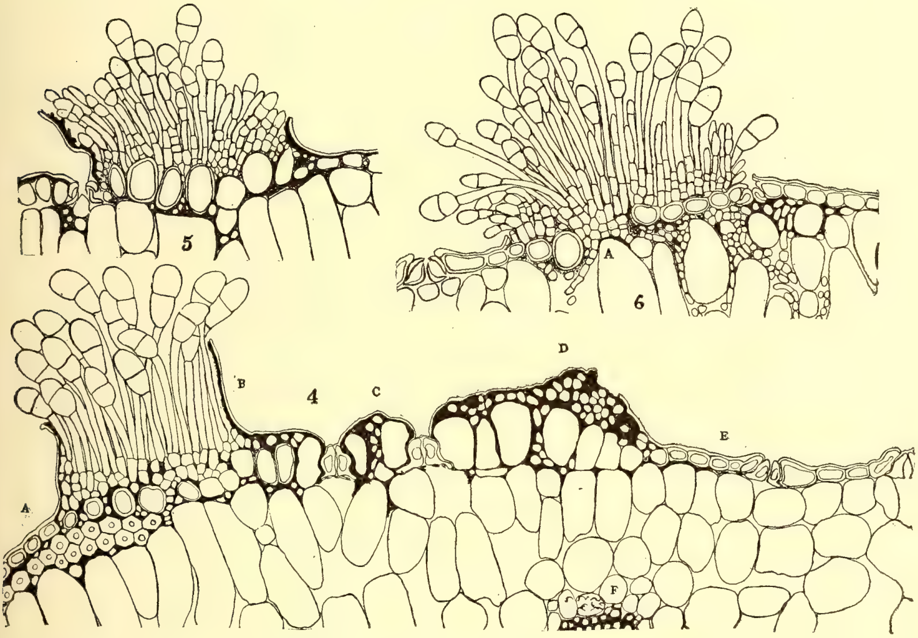


FIG. 4. Part of a cross section of a leaf at the margin, showing a sorus above the epidermal layer, which is continuous. See also text. FIG. 5. Cross section of a portion of leaf through a sorus. The epidermal layer is unbroken, but the cells are swollen. The sorus originates between the cuticle and the epidermis. FIG. 6. Section of the same sorus 70 μ above. Mycelium has pushed in between the epidermal cells at *A*. Very little hypertrophy of the epidermal cells beneath the sorus here.

It sometimes happens, especially in sections cut rather thick, that the portion of the cuticle raised above the sorus, and to which cling some of the disorganized remains of the cutinized walls of the epidermal cells, becomes turned over by pressure on the cover glass so that one is very likely to be misled into thinking that the sorus is really subepidermal or intra-epidermal because what appear to be portions of the epidermis lie above the sorus. The corrosive effect of the hyphae, especially of the buffer cells, on the cuticularized layer is such that indentations or notches are left in the layer which, as noted, clings to the cuticle finally raised above the sorus. These markings are all the more misleading inasmuch as the outlines of the epidermal cells also persist in the raised portion, because the cuticularized part of the walls extends down between the epidermal cells, forming a sort of cap. One can readily show that there are two sets of markings or notches in the portion raised above the sorus by photographing oblique sections of a mound-shaped sorus. Such a surface view is shown in Plate XXII, figure 8. Unquestionably additional nourishment for spore formation may come from the mycelium in the mesophyll, and it is not impossible that hyphae present in this region may push up between the epidermal cells to form the telial stroma in the cuticular layer. Such a sorus would not, however, be subepidermal. The red cedar leaf is strengthened by stereome tissue at the edge as shown below *A* in figure 4. Nothing but epidermal cells lies above this stereome, and the epidermal layer is unbroken under this sorus from *A* to *E*, although at *C* and *D* its cells are hypertrophied.

The Sorus in Young Stems

When young stems are infected, sori appear at the margins of the leaf bases or in the axils. It is unnecessary to describe in detail the development of the sorus, as the epidermis, provided with stomata, persists for two or three years and the sorus is formed in the cuticular layer just as it is in the leaf. It is much more difficult to trace the unbroken epidermis beneath the sorus because of the great irregularity of the cells in the region of the leaf axils where the epidermal cells are normally very large, often pointed, and frequently separated by intercellular spaces. In an infected stem these cells are generally still further enlarged and come to look like cortex cells, especially as they are pushed aside or displaced by the invading strands of mycelium. In no case has there been found the slightest evidence that the sorus is subepidermal. Figure 7 shows a portion of a sorus on a young stem, the raised cuticle carrying a part of the cuticularized wall of the epidermal cells appearing at the right.

It is an important point to determine just where the sorus originates, although it is the morphology of the fungus and not the host-parasite relation that determines its place in a classification. This relationship, however, may very often indicate special adaptations developed in the course of evolution, and as such may be characteristic of a number of species in a given group.

The writer is indebted to Professor J. C. Arthur and Dr. J. F. Adams for criticisms written after an examination of some of the preparations, and in line with their suggestions there has been an attempt to describe still more clearly the exact nature of the origin of the telial sorus of this unique Gymnosporangium.

Prof. Arthur writes that he thinks my use of the term *subcuticular* is misleading and suggests the term *superepidermal* as more accurately describing the location of the telium of this species. After examining a number of species of rusts having "*subcuticular*" sori I am convinced that much further careful work along this line is desirable. It is possible that the introduction of the new term will be found advantageous and even necessary. For example, preparations of young material of *Peridermium Peckii*, *P. balsameum*, *P. Hydrangeae*, and *Caeoma abietis-canadensis*, loaned by Dr. Adams, seem to show that the pycnia of these forms arise in the cuticular layer of the host in the same way that the telium of *Gymnosporangium clavipes* is developed, and that in the first two forms the epidermis entirely disappears beneath the mature pycnium. The pycnia of these forms have always been described as subcuticular,[8] and it is in this broad sense that the term has been used in this paper.

It should have been noted that as the sorus of *G. clavipes* ages the walls of the epidermal cells frequently disappear and the massing of hyphae continues until one can find gaps in sections where no epidermal cells separate the sorus from the mesophyll. This condition is due either to the gradual absorption or crushing down of the original cells or to the wide separation of them as noted previously in connection with figure 6 at *A*.

The Origin of the Teliospore

It was found that the teliospore buds of *G. clavipes* on trunks or cork-covered branches arise from subterminal cells of the basal primordium, agreeing in this respect with the other species previously studied by the writer. It then became a matter of interest to learn how the spores arise in the primary or first sori, which always occur on leaf blades or on young stems still unprovided with cork. The rapid maturation of spore initials, the mucilaginous degeneration products surrounding cells and the cuticularized layer, and the natural tendency of the spore walls to swell, all contribute to make it somewhat difficult to obtain sections showing clearly a series of stages in the origin of the spores.

If one follows the development of the terminal or buffer cells in a very small leaf sorus, he will see that the cuticle is apparently very much stretched and remains unbroken for some time. Fully expanded buffer cells can be found in a sorus where other terminal cells are just beginning to disorganize. Half of a small sorus at the margin of a leaf base on a young stem is shown in figure 7. At *A* the cuticle with the clinging, disorganized cuticularized layer (shaded) is pushed up. Buffer cells are degenerating, and a teliospore bud is arising from a subterminal cell. The remains of the nuclei in the terminal cell at *B* show as deeply stained masses, but the cell still contains some cytoplasm. A young teliospore adjacent to an old buffer cell is shown at *C*. By cutting oblique sections of somewhat larger stems, rows of terminal buffer cells can be found, raising the cuticle for some distances. In still older stems which are provided with a cork layer, the pseudoparenchymatous primordium arises from hyphae mostly confined to the phellogen layer (Pl. XXII, fig. 4). The cork is raised and broken by the buffer tissue in the manner which I have described (*l.c.*) for the effuse trunk forms of *Gymnosporangium nidus-avis*.

---

[8] Ludwig, C. A. Notes on some North American rusts with Caeoma-like sori. Phytopath. **5** : 278. 1915.

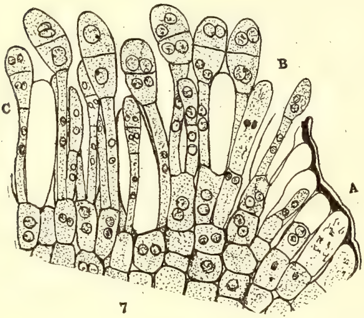
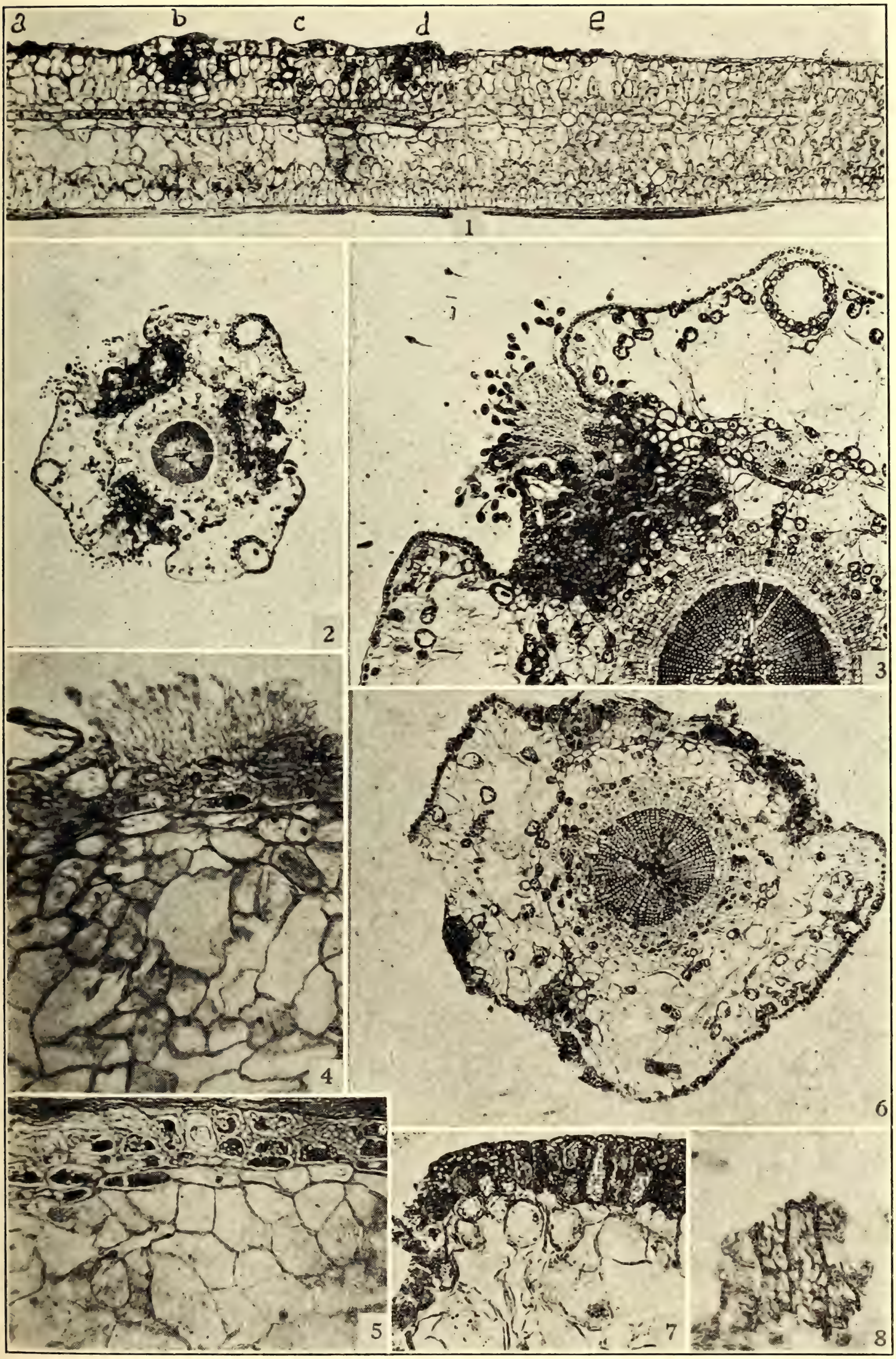


FIG. 7. Buffer cells in a sorus on a young stem at the margin of a leaf. At *A* and *B*, various stages in buffer cell formation. Teliospores arise from subterminal cells. At *C*, a young spore arising from the subterminal cell, above which can still be seen the buffer cell.

However limited may be the attack by the sporophytic stage of this species on Juniperus, it should be noted that it is quite the contrary case with the gametophytic mycelium in Crataegus. Here the hyphae penetrate deeply into the cortex down to the cambium, which loses its identity and ceases to function in an orderly fashion so that stereome and sieve tubes are often entirely wanting on the side of the stem invaded. The outer margin of the wood ring is irregular and indefinite. Haustoria are found in pith rays and in parenchyma cells surrounded by xylem. There seems to be no special tendency to obtain food from cells of the epidermis or phellogen, although in material cut late in the season haustoria are found in nearly every living cell. They are long, irregular, and hypha-like, branching and coiling about in the cell, quite unlike the trim, constantly binucleated haustoria of the sporophytic stage.

In cedar leaves or young stems sporophytic hyphae surround epidermal cells, attacking the cuticularized layers, and sending haustoria even into the guard cells, and it is interesting to find them in cells whose walls have become somewhat suberized. Substances related to those from which cutin is finally formed naturally occur more abundantly in these regions. In older stems provided with a cork tissue, the cells of a phellogen are certainly rich in substances undergoing a series of transformations in the layers of their walls such that by their final elaboration they may be deposited as suberin. These facts noted above might be taken as further evidence that the transition substances entering into cutin and suberin are closely allied. It is not strange, then, that this species is very limited in its attack on the host and that the first sori, *i.e.*, those on leaves and young stems, are subcuticular. A fundamental morphological feature such as the formation of buffer cells from the terminal cells in the sorus primordium is one fixed in the course of evolution and not to be easily affected by environmental conditions. Further cytological study will undoubtedly result in the dis-


a
b
c
d
e
1
2
3
4
6
5
7
8

DODGE: THE GENUS GYMNOSPORANGIUM

covery of other rusts outside this genus whose teliospores arise from subterminal cells.

SUMMARY

The sporophytic mycelium of *Gymnosporangium clavipes* on *Juniperus virginiana* in the periods of exploration and attack is found in the cuticularized layer of the epidermal cells of the leaves and young stems. As the season advances the hyphae penetrate into the mesophyll. In cork-covered stems the parasite is generally confined to the two or three outermost layers of cells of the living cortex, although hyphae are sometimes found much nearer the phloem. Characteristic binucleated haustoria occur in the cells of the epidermis and mesophyll.

The first sori always appear either directly on the leaves or, more commonly, on the stems at the margins of the decurrent leaf bases or in the leaf axils. In later years, as cork is laid down on the stem, the sori are formed and break through in the ordinary manner.

Sori on leaves and young stems are subcuticular to the extent that they arise in the cuticularized layer of the epidermal cells.

The teliospore buds grow out from the subterminal cells of the basal primordium, the terminal cells having become disorganized and swollen, functioning as buffer cells.

FRUIT DISEASE INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY

EXPLANATION OF PLATE XXII

Gymnosporangium clavipes

FIG. 1. Longitudinal section of a part of a leaf at the edge of the vein. The mycelium is found principally in the upper epidermis from *a* to *e*. At *b*, *c*, and *d*, hyphae have invaded the mesophyll as shown by the darker regions.

FIG. 2. Section of infected branch of red cedar showing three regions containing mycelium.

FIG. 3. More highly magnified view of a portion of a similar section. The dark area between the leaf bases shows the limits of penetration.

FIG. 4. Section of a sorus on a larger limb; the mycelium beneath is confined to the region of the cork cambium. A fragment of the ruptured cork is seen at the left.

FIG. 5. Section from an infected area between two sori; the mycelium is likewise very superficial.

FIG. 6. Section of an infected stem similar to the one shown in figure 2, but made January 19th, the following winter. The old infected tissue has been cut off by wound callus (center above), and hyphae have grown in from the sides, attacking the cork cambium present only between the two leaf bases.

FIG. 7. Cross section at the edge of an infected leaf. The dark region indicates where the fungus is present. Cross sections of the hyphae are best shown in the cuticular layer.

FIG. 8. Surface view of a fragment of the cuticle with attached cuticularized layer raised above the sorus. Two kinds of markings: the outlines of the epidermal cells, and the corrosion pockets left by the hyphae or buffer cells of the sorus.

CERTAIN RELATIONS BETWEEN ROOT DEVELOPMENT AND TILLERING IN WHEAT: SIGNIFICANCE IN THE PRODUCTION OF HIGH-PROTEIN WHEAT

W. F. Gericke

(Received for publication December 7, 1921)

In two other papers,[1] the writer has shown how differences in the protein content and in the consequent quality of a "soft white" spring wheat (White Australian) are causally related to the nitrogen nutrition of the wheat plant. The conditions that made for the production of high-protein wheat in the investigation cited arose from applications of nitrogen at much later growth phases of the plant than that indicated by the seeding or early seedling stage. Abundant tillering and culm production were obtained from the cultures which received nitrogen late in the growing period, that is, from 48 to 110 days after planting. The cultures that received nitrogen at the time of planting tillered sparsely and produced fewer culms, usually only one per plant.

That such marked differences in tillering could be obtained from equivalent applications of nitrogen per culture, applied, however, at different growth periods of the plant, suggested further study as to the causal conditions for these results. The cultures that produced the high-protein wheat also produced a much larger amount of total dry matter, this being partly accounted for by the abundant tillering. Obviously, this fact would suggest that the high-protein wheat cultures absorbed and utilized more nitrogen than the plants which produced the low-protein wheat, and this in view of the facts that all the cultures received the same amounts of nitrogen and that those that had the application of nitrogen during the longest period of time absorbed less than did those that had it for a much shorter period.

That these differences in the amount of nitrogen absorbed by the plant may be accounted for by conditions arising out of differences in the extent of the root development of the cultures at the time nitrogen was supplied seemed to be a plausible explanation. It is generally known, and was also observed by the writer in the investigation cited, that the root development of wheat (and the same seems to hold for many other plants) is decidedly affected by the supply of nutrients in the soil in which the plants are rooted. The quantitative relation, however, of differences in the extent of the root development of a plant to the extent of its top, when grown under different sets of conditions, has not been sufficiently studied. Especially is this true in soil cultures, where it is difficult to make exact determinations. It is conceivable that the kind of root development a plant may have when a treatment such as an application of nitrogen is made to it may be a very important factor that affects materially the nature of the subsequent responses of the plant.

---

[1] On the protein content of wheat. Science, n. ser. **52** : 446, 447. 1920. Certain relations between the protein content of wheat and the length of the growing period of the head-bearing stalks. Soil Sci. **13**: 135–138. 1922.

In order to obtain data as to what effect differences in the extent of root development of wheat seedlings would have upon the tillering of the plants when grown in the same nutrient solutions, the following experiment with water cultures was carried out: Wheat seedlings 8 to 10 centimeters high, having roots 10 to 12 centimeters long, were set up according to the usual method employed for solution-culture investigations, and were grown for 25 days in two-quart containers filled with tap water from the laboratory. At the end of this period, the seedlings had developed a very extensive root system. Roots from 50 to 70 centimeters long had formed while the tops had grown only a little, attaining a height of approximately 10 to 12 centimeters. Sets of these cultures with large root development were then transferred, respectively, to several different kinds of complete nutrient solutions. At the time these several sets were placed in the nutrient solutions, other sets of wheat seedlings a few days old having shoots 8 to 10 centimeters high and a comparatively small root development, *viz.*, roots about 10 to 12 centimeters long (similar to those used for the tap-water series), were set up to serve as controls, being placed in similar nutrient solutions to those in which were placed the sets of large root development. The investigation, therefore, as planned, concerned itself with a study of some of the effects the same kind of nutrient solution would have upon wheat seedlings of different root development, some having a large root development and others a comparatively small root development at the time the cultures were placed in the nutrient solutions. In one class of cultures, practically one half of the total dry weight was contained in the roots, while in the other class about one fourth of the total dry matter was roots. The roots of the cultures grown in tap water for 25 days were from four to five times as long as their tops. The lengths of the roots of the plants not so treated were only a little greater than those of their tops. The weight of the tops of the two sets of cultures was about the same, but the weight of the roots in the one was about four times the weight of the other. Subsequent treatment of these two classes of cultures was the same throughout the test period employed. The data in table 1 are given as an example of the results obtained:

TABLE 1. *Effect of Differences in Extent of Root Development of Wheat Seedlings upon Tillering*

Average of 10 cultures (5 seedlings per culture) grown 4 weeks in nutrient solution

	Length of Tops (cm.)	Length of Roots (cm.)	Weight of Tops (g.)	Weight of Roots (g.)	No. of Tillers per Plant
Cultures grown in tap water 25 days after seedlings were set in corks......	11	62	0.19	0.18	5.4
Cultures transferred directly to nutrient solutions at time seedlings were set in corks......................	10	12	0.17	0.045	1.2

It will be noted from the table that the cultures of large root development tillered much more profusely than did those cultures which did not have large root development at the time they were placed in the nutrient solution. The question may now be asked as to the cause of the profuse tillering in the one case and of its lack in the other. The answer seems to be that in one case the cultures absorbed a greater amount of nutrients from the solution than they did in the other case. The cultures of large root development absorbed a much larger amount of nutrient from the solution because they had a larger root area with which nutrients came in contact. The profuse tillering of these cultures with the large root development, therefore, may presumably be accounted for by the fact that the cultures took up more nutrient than was needed by the plants for the normal development of the individual shoots of the seedlings. The consequence was a great vegetative response in the form of tillers which arose from the root crown. The cultures with the small but normal root development, while they produced some tillers, nevertheless did not produce them in any way comparable in number to those produced by the cultures with large root development. Presumably because of the smaller root development in these cultures, an excess of nutrients was not absorbed by these seedlings, and the condition that made for the profuse tillering above stated did not prevail. Although the cultures of large root development tillered much more profusely in all the different nutrient solutions used than did those of relatively small root development, nevertheless the chemical properties of the nutrient solution itself, as indicated by different concentrations of certain salts used, were also an important factor that affected tillering. This was shown by the fact that cultures of similar root development differed in the number of tillers produced, depending upon the kind of nutrient solution in which the seedlings were placed.

It seems that data obtained with these solution cultures show why good tillering was obtained with the soil cultures referred to in the papers cited. As stated, the soil used in that experiment was low in nitrogen, so that the conditions of the growing medium in respect to the paucity of this element (nitrogen) may be compared to that existing in tap water. The wheat seedlings grown in the nitrogen-poor soil developed a large root system after a period of several weeks. When nitrogen was added to these cultures, relatively large amounts of this nutrient were absorbed by the plant. This was more than was needed for the normal growth of the single shoots these seedlings had. The result from this treatment was a renewal and stimulation of vegetative growth. This gave rise to abundant tillering, which followed soon after the application of nitrogen. The cultures that received nitrogen at the time of planting obviously did not grow in a nitrogen-poor soil and consequently did not develop the same kind of root system as did those cultures grown in a nitrogen-poor soil.

That the large root development in proportion to that of tops obtained in the cultures grown in tap water was primarily due to the deficiency of nitrogen, was further substantiated by the fact that this peculiar large root development was obtained with wheat seedlings grown in several different kinds of nitrogen-free "nutrient solutions" prepared from different kinds of salts. This, however, does not mean that a nitrogen-poor medium is the only condition that may make for an abnormally large root development in wheat seedlings or in other plants. .

It seems that the results obtained from these simple tests show that the extent of the root development of the wheat seedlings when nutrients are made available or become so is a matter of importance in the economy and culture of this important food plant.

Laboratory of Soil Chemistry and Bacteriology,
University of California

THE POLLINATION OF MARCGRAVIA: A CLASSICAL CASE OF ORNITHOPHILY?

Irving W. Bailey

(Received for publication December 12, 1921)

Introduction

Since the publication of Darwin's investigations upon the pollination of orchids, many biologists have tended to assume that flowers which are visited by insects and birds are pollinated by these animals, and that aberrant types of floral organization are adaptations to insure cross-pollination. Thus, the bizarre inflorescences of Marcgravia, with their curious pitcher-like nectaries, are assumed to facilitate cross-pollination by birds. In fact, this extraordinary genus is cited as one of the most striking illustrations of ornithophily.

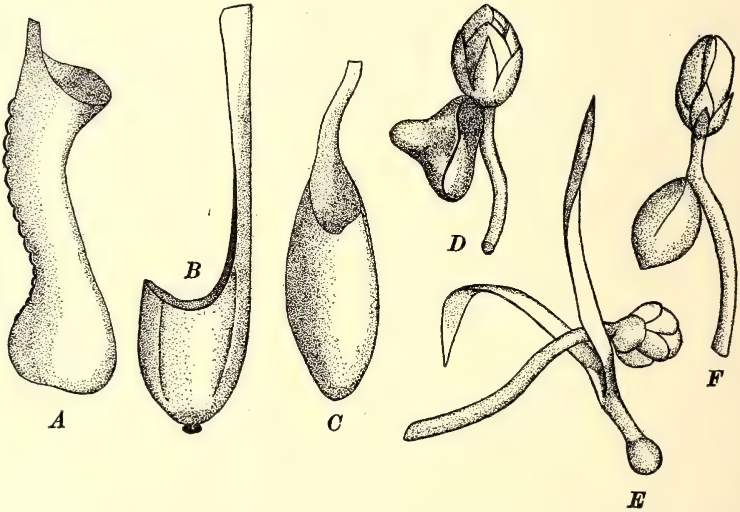


Fig. 1. Nectariferous appendages of Marcgraviaceae. *A. Marcgravia coriacea* Vahl. *B. M. picta* Willd. *C. Norantea guianensis* Aubl. *D. Souroubea pilophora* Wittm. *E. S. guianensis* Aubl. *F. Ruyschia sphaerodenia* Delp. After Wittmack.

While collecting along the banks of the Mazaruni and Cuyuni Rivers near the Tropical Station of the New York Zoölogical Society,[1] the writer encountered two species of Marcgravia. As much time as could conveniently be spared from other work was devoted to a study of the structure and pollination of these interesting plants. Before discussing the results of this investigation, however, it is essential to outline certain salient morphological features of the Marcgraviaceae.

---

[1] At Kartabo, British Guiana.

SALIENT FEATURES IN THE MORPHOLOGY OF THE MARCGRAVIACEAE

As shown by Jussieu (1809), Delpino (1869), Wittmack (1878), Szyszylowicz (1895), and others, the small neotropical family Marcgraviaceae is characterized by having nectariferous appendages which are closely associated with the flowers. These nectaries vary considerably in size, shape, and structure in different species and genera, and are significant in the classification of the various representatives of the family (text fig. 1). In the genera Caracasia and Ruyschia, they are small spherical or hemispherical organs which are attached to the pedicels of the flowers, as are the more or less deeply concaved and spurred nectaries of Souroubea. In Marcgravia, on the contrary, they are relatively large sac-shaped or galeate structures which are inserted upon the apex of the peduncle.

There has been considerable speculation concerning the origin and morphological significance of these nectariferous appendages. Are they metamorphosed bracteoles, abnormal pedicels, modified bracts, or appendages *sui generis?* Most recent students of the Marcgraviaceae have accepted Planchon and Triana's (1863) conclusion that they are evaginated bracts. It must be admitted that there is considerable evidence in favor of this view.

The leaves of the Marcgraviaceae are provided with hypophyllous excretory organs which vary considerably in size, number, and distribution in different representatives of the family. Two of these glands tend to be located at the base of the lamina, one on either side of the midrib. In small, rudimentary leaves, such as commonly occur near floral axes, these basal glands become proportionately accentuated as the lamina is reduced in area. Interesting transitions between rudimentary glandular leaves of this type and spoon-shaped, hooded, or sac-shaped nectaries occur in various Marcgraviaceae; and are particularly numerous and conspicuous in Norantea, *e.g.*, *N. anomala* H.B.K. and *N. brasiliensis* Choisy. In certain specimens, the nectariferous bracts at the base of the inflorescence resemble rudimentary leaves and are attached to the peduncle just below the points of insertion of the pedicels, but in the middle and upper portions of the racemes they become more and more deeply concaved or evaginated, and their petioles fuse with, and therefore appear to arise from, the pedicels of the flowers. The concave or inner surface of the nectariferous appendages is the morphological equivalent of the under surface of the leaves. The outlets or pores of the hypophyllous glands are located in this dorsal surface (Pl. XXIII, fig. 2) and, accordingly, discharge their sugary excretions into the concavities of the bracts (Pl. XXIV, fig. 9).

In the more or less elongated, usually erect, racemes of Ruyschia, Caracasia, Souroubea, and Norantea, there is a nectariferous bract for every flower; whereas in the pendent, more or less compacted, umbelliform racemes of Marcgravia, the nectaries are segregated at, and apparently arise from, the terminal portion of the peduncle, which usually is devoid of fertile pedicels (text figs. 2, 4). These nectariferous bracts are not exact morphological equivalents of those which occur in the other genera, for they bear rudimentary flower buds at their apices (text figs. 3, 5). That the nectaries of Marcgravia are not abnormal pedicels, as maintained

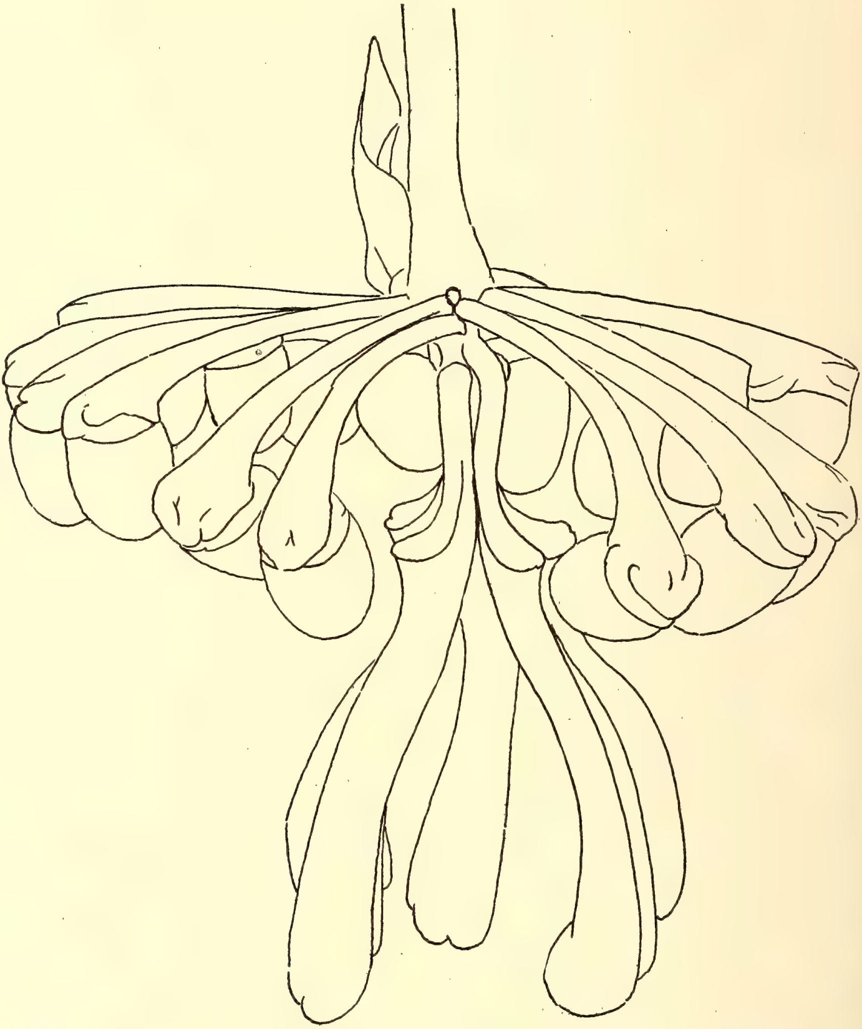


FIG. 2. *Marcgravia cuyuniensis* spec. nov. Mature inflorescence. One fertile pedicel removed from in front to show attachment of nectaries. From a field sketch by Miss Anna H. Taylor. × 1/1.

by Seemann (1870), but are compound structures resulting from the fusion of a nectariferous bract and a sterile pedicel, is indicated, not only by their external morphology, but also by their internal anatomy. The fused pedicel shows as an embossed rib and terminates in a rudimentary flower bud, which occasionally develops into a normal flower (text figs. 3, 5). The fertile pedicels commonly are provided with corky excrescences. The outer surfaces of the adnate sterile pedicels are also conspicuously verrucose. Furthermore, as shown in figure 6, Plate XXIV, there are two distinct systems of fibro-vascular bundles in the nectaries, one belonging to the sterile pedicel and the other to the adnate bract.

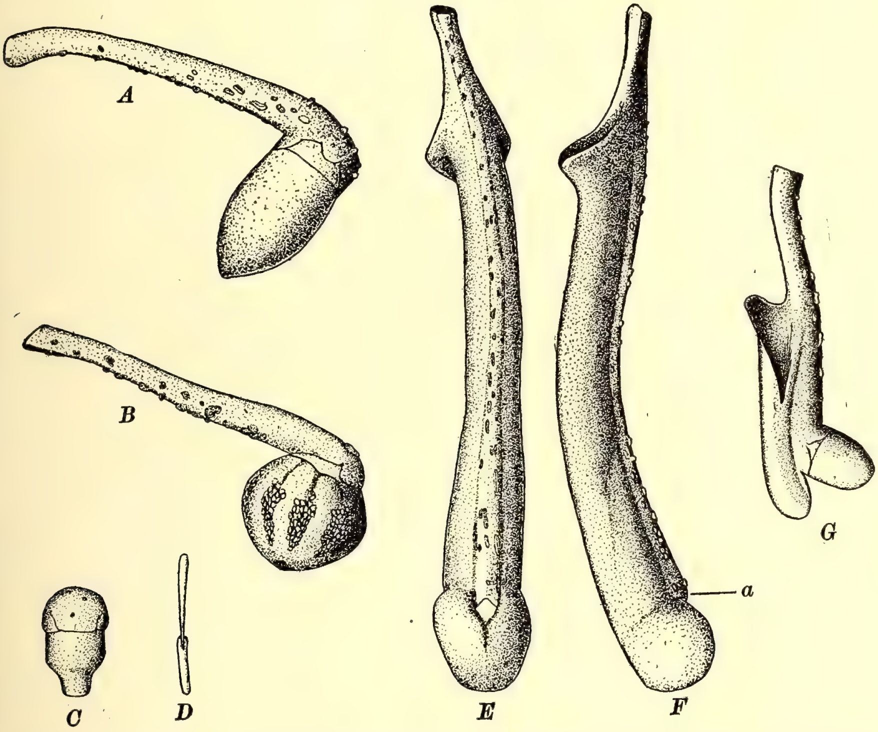


FIG. 3. *Marcgravia cuyuniensis* spec. nov. *A*. Pedicel and flower bud. *B*. Pedicel and fruit. *C*. Pistil. *D*. Stamen. *E*, *F*. Nectariferous appendages; (*a*) rudimentary flower bud at apex of adnate pedicel. *G*. Abnormal appendage, showing adnate flowering pedicel. × 1/1. Drawn from material preserved in formalin-alcohol by Miss Grace Griffin.

Although there are traces of cohesion of floral members in Ruyschia, Souroubea, and Norantea, the flowers of Marcgravia are characterized by having calyptriform corollas. The petals are fused into a tough, leathery capsule or thalamus which entirely encloses the pistil and stamens (text fig. 4). This capsule does not split longitudinally at the time of "flowering," but becomes detached at its attenuated base. Delpino (1869) divided the genus Marcgravia into two subgenera: Orthothalamium, having the capsules in line with projections of the pedicels, and Plagiothalamium, having them turned downwards at right angles to the pedicels.

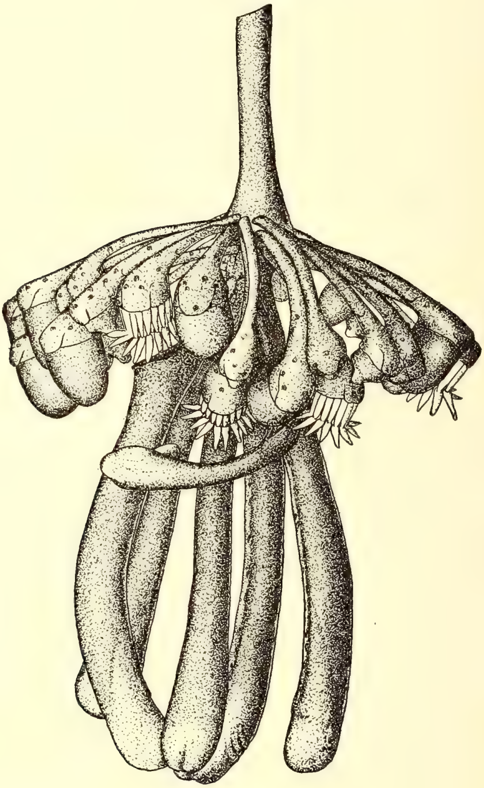


FIG. 4. *Marcgravia purpurea* spec. nov. Mature inflorescence. Four calyptriform corollas removed to show stamens. × 1/1. Drawn from material preserved in formalin-alcohol by Miss Grace Griffin.

It is evident, accordingly, in viewing the Marcgraviaceae as a whole, that there are certain distinct and closely correlated lines of morphological specialization which reach their climax in Plagiothalamium. In this subgenus the racemes are compacted into pendulous umbels, the large, highly modified nectariferous bracts are terminally segregated and are adnate to sterile pedicels, the corolla is fused into a deciduous capsule or thalamus, and the flower buds are bent downwards at right angles to the pedicels.

SIGNIFICANCE OF FLORAL MORPHOLOGY OF MARCGRAVIACEAE

Delpino (1869), who devoted considerable attention to the Marcgraviaceae, believed that the nectariferous bracts function as attracting organs in connection with the cross-pollination of the protandrous flowers; the small nectaries providing food for insects, and the larger and more complex nectariferous appendages of Souroubea, Marcgravia, and certain species of Norantea supplying a delectable beverage for birds.

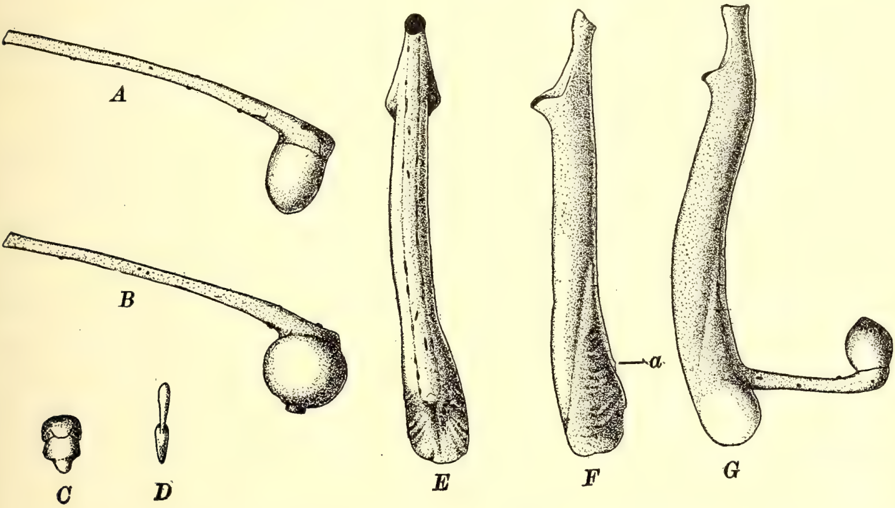


FIG. 5. *Marcgravia purpurea* spec. nov. *A*. Pedicel and flower bud. *B*. Pedicel and fruit. *C*. Pistil. *D*. Stamen. *E*, *F*. Nectariferous appendages; (*a*) rudimentary flower bud at apex of adnate pedicel. *G*. Abnormal appendage, showing elongated pedicel and flower bud. × 1/1. Drawn from material preserved in formalin-alcohol by Miss Grace Griffin.

Belt (1874) was the first, however, to offer any concrete suggestion concerning the significance of the morphological peculiarities of the highly specialized inflorescences of Marcgravia. The following paragraph, quoted from his highly entertaining book, "The Naturalist in Nicaragua," summarizes his hypothesis:

> Higher up the valley more trees were left standing and amongst these small flocks of other birds might often be found, one green with red head (*Calliste laviniae* Cass.); another shining green, with black head (*Chlorophanes guatemalensis*); and a third, beautiful black, blue and yellow, with a yellow head (*Calliste larvata* Du Bus.). These and many others were certain to be found where the climbing *Marcgravia nepenthoides* expanded its curious flowers. The flowers of this lofty climber are disposed in a circle, hanging downwards, like an inverted candelabrum. From the center of the circle of flowers is suspended a number of pitcher-like vessels which, when the flowers expand, in February and March, are filled with a sweetish liquid. This liquid attracts insects, and the insects numerous insectivorous birds, including the species I have mentioned and many kinds of humming-birds. The flowers are so disposed with the stamens hanging downwards, that the birds, to get at the pitchers, must brush against them, and thus convey the pollen from one plant to another. A second species of Marcgravia, that I found in the woods around Santo Domingo, has the pitchers placed close to the pedicels of the flowers, so that the birds must approach them from above; and in this species the flowers are turned upwards, and the pollen is brushed off by the breast of the birds.

Belt's generalization, that the inflorescences of Marcgravia are adaptations to insure cross-pollination by birds, is so plausible and appears to afford a satisfactory explanation for so many closely coördinated phenomena that it is not surprising that it should have been accepted without question by Hermann Müller (1873), Schimper (1898),[2] and others; particularly in view of the fact that the Marcgraviaceae are stated to be protandrous.

Descriptive and Taxonomic

The two species of Marcgravia growing in the vicinity of the Kartabo laboratory are characterized, as are other representatives of the genus, by having two distinct types of branches: (1) sterile runners (plagiotropic), and (2) pendulous fertile shoots (orthotropic) which bear terminal inflorescences. The former are provided with numerous clasping roots and small distichous leaves, whereas the latter have large distichous leaves. The plants scramble over the trunks and lower branches of trees which line the banks of the larger water courses. They are not lofty climbers, rarely rising more than fifteen or twenty feet above the surface of the rivers. Indeed, at high water certain of the inflorescences and clusters of fruit are completely submerged.

The two species may readily be distinguished, even at a considerable distance, by conspicuous differences in color and habit of growth. In one species, which was designated in the field as *A*, the fertile shoots are relatively infrequent, relatively long, and bear dark green leaves which are so oriented that their upper surfaces are clearly visible (Pl. XXIII, figs. 1, 3). In the other species, *B*, the flower-bearing shoots are more numerous, shorter, and have yellowish-green leaves that are nearly horizontal and are considerably folded dorsally (fig. 4). Upon closer inspection the broadly oblong-elliptical leaves of species *A* are found to be strongly petiolate and to be inserted upon a zigzaged axis (fig. 1), whereas those of species *B*, which are smaller and narrower, are nearly sessile and are borne on a straight shoot. Furthermore, the yellowish or brownish-green inflorescences of the latter species are attached close to the last leaf, whereas in the former species the dark, greenish-purple inflorescences form the terminus of a long, straight, terete peduncle.[3] As shown in text figures 2–5, the flower buds, fruits, pedicels, and nectaries of species *B* are larger and stouter than homologous members of the inflorescences of species *A*. The vines of the former bear inflorescences and fruits in various stages of maturity, whereas the fertile shoots of species *A*, at any given time, are all in equivalent stages of differentiation. In other words, species *B* flowers continuously, but species *A* exhibits a marked floral periodicity.

[2] Schimper, in quoting the above passage from Belt, substitutes *Marcgravia umbellata* for *Marcgravia nepenthoides*. He also figures the inflorescence of the former instead of the latter species.

[3] During the earlier stages in the development of the flowering shoots of species *A*, the peduncle may be provided with numerous small glandular leaves. Most of these more or less rudimentary leaves drop off before the inflorescences attain any considerable size.

Concerning the identity of these Marcgravias, it may be stated at once that they both belong in Delpino's subgenus, Plagiothalamium. Furthermore, the prolongation of the peduncle beyond the points of attachment of the pedicels in species *B* indicates clearly that this species should be referred to Wittmack's subsection Dolichoracheae, just as the absence of this extension in species *A* leads to its inclusion in his Brachyracheae.

The leaves and inflorescences of Marcgravias vary considerably, particularly in different stages of their ontogeny, and are difficult to season for herbarium purposes. Many of the descriptions of species in the literature appear to be based upon the study of somewhat fragmentary material; not infrequently of immature leaves or inflorescences. Such facts as these, coupled with the difficulty of securing access to the widely scattered type specimens, makes the determination of species a somewhat difficult and unsatisfactory undertaking.

Species *B* appears to be closely allied to *M. coriacea* Vahl and *M. acuminata* Miguel, but differs from these species in certain of its floral and foliar characters, particularly in having filaments which are not basally connate. Species *A* resembles *M. parviflora* Rich. var. *pedunculosa* (Triana and Planchon) Wittm., but has large nectaries which are longer than the pedicels of the flowers. In view of the abundance of both living and dried material at my disposal, it seems wiser to describe these plants as new species, rather than to refer them to any of the above-named species. Furthermore, it is desirable to make detailed and fairly comprehensive descriptions, since there appears to be some doubt as to which characters are of greatest specific significance in these sections of Marcgravia.

Marcgravia (Dolichoracheae) **cuyuniensis** spec. nov.

Folia ramorum floralium breviter petiolata, oblongo-elliptica, coriacea, ex glandulis marginalibus depressis numerosis subcrenulata, apice acuminata, basi acuta vel subrotundata; in sicco haud nitentia, supra punctata, venis patentibus; glandulis hypophyllis saepissime duobus, ad basim costae mediae; nervo crasso subtus prominente, supra basim versus profunde sulcato; petiolis crassis antice canaliculatis. Racemus umbelliformis, pendulus, submultiflorus, breviter pedunculatus. Bracteae 3–6, elongatae, tubuloso-cucullatae, crassae, clavatae, deciduae, sub ore rotundato plus minusve constrictae. Pedicelli 16–24, crassi, tuberculato-verrucosi. Corolla coriacea, ovoidea vel conico-ovoidea. Stamina ± 46, *filamentis basi non connatis*, antheris rubellis. Ovarium 9- — 11-loculare. Fructus magnus, globosus, rubidus, plus minusve tuberculato-verrucosus, stigmate mammiformi coronatus. Semina sanguinea nitentia

Scandent epiphytic shrub. Plagiotropic shoots quadrangular, bearing small coriaceous leaves and short clasping roots. The former oval, oval-oblong, or oblong, distichous, conspicuously glandular at the base. The latter aggregated in clusters, more or less completely sheltered by the leaves. Orthotropic fertile shoots numerous, short, stout, usually terete and pendulous, sparsely linear-verrucose. Leaves distichous, coriaceous, somewhat conduplicate dorsally, elliptical-oblong, acuminate, acute, retuse, or subcordate at the base, 10–16 cm. long, 3.5–4.5 cm. broad, glabrous, glossy, yellowish green with midrib and principal veins outlined in yellow; when dry, dull, scabrous, punctate above, veins more or less prominent and embossed; petiole 3–5 mm. long, canaliculate above, subtending a long, shallow groove in the stem; two basal glands large, conspicuous; glandular marginal depressions numerous, making the leaves distinctly subcrenulate. Umbelliform racemes yellowish or brownish green, pendulous. Pedicels stout, horizontal or curved downwards at the time of flowering, 4.5–5.5 cm. long, covered with large, conspicuous, corky excrescences. Calyptriform corolla thick, coriaceous, ovoid or ovoid-conical, 1.3–1.8 cm. long, 1.0–1.2 cm. diameter. Stamens numerous, free; filaments cream-colored, 8–11 mm. long, tapering, stout and more or less quadrangular at the base; anthers reddish or pink at maturity, lanceolate, 5–7 mm. long. Ovary slightly obovoid, ± 7 mm. diameter, 9- to 11-locular, abruptly conical above, terminating in a short, blunt style, ± 3 mm. long. Fruit large, globose, ± 2.0 cm. diameter, crowned by the slightly elevated hemispherical stigmatic surface; at maturity reddish, scabrous or banded-verrucose. Seeds glistening, blood-red. Nectaries elongated, 5.5–8.0 cm., considerably swollen at the apex, tapering towards the base; orifice surrounded by a protruding rim or lip; petiole ± 15 mm. long; fused pedicel showing as a slightly embossed, verrucose rib; flower bud rudimentary, depressed.

Banks of the Cuyuni River near Kartabo, British Guiana. Bailey nos. 128, 177, 178, and 193, deposited in the Gray Herbarium of Harvard University.

Marcgravia (Brachyracheae) **purpurea** spec. nov.

Folia ramorum floralium pro genere longe petiolata, elliptica vel oblongo-elliptica, coriacea, basi acuta, apice acuminata vel attenuato-acuminata; in sicco membranacea, supra punctata, venis secundariis patentibus; nervo crasso supra sulcata, petiolis crassis antice canaliculatis; glandulis hypophyllis inconspicuis, duobus, ad basim costae mediae; glandulis marginalibus depressis paucis. Racemus umbelliformis, multiflorus, purpureus, longius pedunculatus. Bracteae 4.7, elongatae, tubuloso-cucullatae, *pedicellis longiores*, clavatae, deciduae. Pedicelli 24–42, graciles, pauce tuberculato-verruculosi, prophyllis a calyce discretis. Corolla conico-ovoidea, purpurea. Stamina ± 16, libera; antheris rubellis. Ovarium turbinato-globosum in stigma productum, 7- - 8-loculare. Fructus parvus, globosus, stigmate apiculo coronatus.

Scandent epiphytic shrub. Plagiotropic shoots quadrangular, bearing small subcoriaceous or membranous leaves and short clasping roots. The former oblong or oblong-oval, distichous, glandular at the base. The latter aggregated in clusters, sheltered by the leaves. Orthotropic, fertile shoots long, bilaterally compressed, flexuous, terminating in a straight, terete peduncle, 15–30 cm. long. Leaves distichous, coriaceous, elliptical or oblong-elliptical, acuminate or attenuate-acuminate, acute at the base, glossy, dark green, 12–19 cm. long, 5–8 cm. broad; when dry, membranaceous with conspicuously embossed veins and veinlets, punctate above; petiole 1.0–1.5 cm. long, deeply canaliculate above; basal glands inconspicuous; marginal glandular depressions widely spaced. Leaves of peduncle 1–6, more or less rudimentary and early deciduous, conspicuously glandular, broadly elliptical or obovate, 5.5–8.5 cm. long, 3.5–5.5 cm. broad, or small oblong or ovate, 2.5–4.0 cm. long, 1.5–2.5 cm. broad. Umbelliform racemes dark greenish purple, pendulous. Pedicels numerous, slender, 3.5–4.0 cm. long, regularly and evenly distributed, straight and nearly horizontal or curved downwards at time of flowering; corky excrescences few, inconspicuous; bracteoles widely separated from the calyx. Calyptriform corolla ovoid or ovoid-conical, thin, coriaceous, 8–11 mm. long, 7–9 mm. diameter. Stamens non-coherent; filaments stout, short, ± 5 mm., quadrangular, abruptly tapering above; anthers broadly lanceolate, reddish, ± 5 mm. long. Ovary turbinate-obovoid, ± 3.5 mm. diameter, abruptly tapering into a short, blunt style, 2.5 mm. long. Fruit small, globose, ± 10 mm. diameter, reddish, scabrous; style and stigma conspicuously protuberant. Nectaries elongated, 5.5–6.5 cm. long, bilaterally compressed, swollen at the apex, tapering towards the base, orifice surrounded by a slightly protruding rim or lip; petiole ± 7 mm. long; fused pedicel showing as a slightly embossed, verrucose rib; flower bud rudimentary, depressed or protuberant.

Banks of the Mazaruni and Cuyuni Rivers near Kartabo, British Guiana. Bailey nos. 136, 179, 192, 195, deposited in the Gray Herbarium of Harvard University.

Pollination of M. cuyuniensis and M. purpurea

(1)

I first was led to question the accuracy of Belt's generalization concerning the pollination of Marcgravia by a detailed study of the arrangement of the nectaries and flower buds in *M. cuyuniensis* and *M. purpurea*. In these species, as in *M. umbellata* L. and most representatives of the Brachyracheae, the pitchers are placed close to the pedicels of the flowers (text figs. 2, 4). Therefore, according to Belt's hypothesis, birds would have to visit these inflorescences from *above*, but the flower buds and flowers are not turned *upwards*.

During my stay at Kartabo, I did not succeed in finding birds in the vicinity of the inflorescences of *M. cuyuniensis*, although many of the nectaries contained a sweetish liquid. Dr. and Mrs. Alfred Emerson and Miss Anna H. Taylor were more fortunate in the case of *M. purpurea*, which flowered after my return to the United States. A humming bird was seen to hover *above* an inflorescence and to sip nectar from one of the pitchers. The following observations of Miss Bryant (1905) are also significant in this connection. She states concerning *M. umbellata* L. (?):

> The plant is common here climbing to the summit of the forest trees, and is frequently visited by humming birds. The bird settles on the *top* of the flowers (inflorescences) and inserts its long curved beak into the pitchers below.

Such observations as these suggested, of course, that the highly specialized inflorescences of these Marcgravias are not efficient mechanisms for insuring cross-pollination by birds.

(2)

During several weeks spent in exploring the banks of the Cuyuni River, I was considerably puzzled by the fact that, although the vines of *M. cuyuniensis* bore flower buds and fruits in various stages of maturity, there appeared to be none that was actually in flower. A more intensive search led to the finding of numerous inflorescences from which the calyptriform corollas had recently fallen, but still no flowering pedicels were in evidence. Finally, an inflorescence was encountered, one evening, which had shed only three of its numerous protecting capsules. Early the next morning nine more were missing, which demonstrated conclusively that the plant had flowered at night. At 11 P.M. the next night five of the remaining buds were found to be in "flower," with their pink stamens still attached at the base of the pistil. It seemed to be highly improbable, therefore, that *M. cuyuniensis* is cross-pollinated by birds, Trochilidae, Coerebidae, or Tanagridae.

(3)

The question suggested itself, accordingly, are the flowers cross-pollinated by moths, bats, or other night-flying animals? In order to throw some light upon this point, several inflorescences were entirely enclosed in specially prepared cloth bags, and several others were divested of their nectariferous appendages. These inflorescences subsequently flowered and produced fruits. But how could the flowers form fruits if the Marcgraviaceae are protandrous, as maintained by Delpino and others? Obviously there were three possibilities to be considered in this connection: (*a*) that the flowers were cross-pollinated by small insects which worked their way into the bags through small openings; (*b*) that the fruits were abnormal and did not contain viable seeds; and (*c*) that the flowers were self-fertile. The first supposition did not appear to be a particularly reasonable one, since certain of the inflorescences (both of the bagged and unbagged specimens) were without their hypothetical attracting organs, *i.e.*, nectaries. Furthermore, when certain of the inflorescences were in flower, there were no other flowering vines within a radius of several miles.[4]

A detailed study of numerous inflorescences at different stages of maturity revealed the fact that dehiscence occurs within the calyptriform corolla. The stamens are so arranged (Pl. XXIV, fig. 7) that the stigma is coated with a thick layer of viscous pollen before the protecting capsule falls off. This layer of pollen persists, and may be found adhering to the stigmatic surface on old fruits. Stamens examined just after the corollas have dropped are found to be more or less completely devoid of pollen and rapidly to become deciduous. As shown in figure 10, the multilocular ovary contains a large number of ovules, only a portion of which produce embryos (figs. 5, 8). The remaining ovules, presumably the unfertilized portion, undergo a curious metamorphosis or process of enlargement. Their outer integument becomes greatly thickened by radial elongation of its constituent cells, which contain a reddish or amber-colored, amorphous substance (fig. 8). The proportion of these aborted ovules varied considerably in different fruits, but was no higher in the bagged specimens ,than in those which were unprotected. In fact, the former frequently contained more embryos or viable seeds than the latter. In other words, *M. cuyuniensis*, instead of being protandrous and cross-pollinated, appears to be self-pollinated and practically cleistogamous.

---

[4] The specimens of *M. cuyuniensis* occurred at infrequent intervals along the widely separated banks of the Cuyuni River, so that there were only a comparatively limited number of individuals in the vicinity of Kartabo. All these plants were carefully located and visited every morning and evening. By observing the deciduous corollas it was possible to determine what vines had flowered during a particular period.

(4)

The objection may be raised at this point that, in dealing with *M. cuyuniensis*, I was concerned with an abnormal or aberrant representative of the genus Marcgravia, and that the typical, day-flowering species are protandrous and cross-pollinated by birds. *Marcgravia purpurea*, which belongs in an entirely different section of the genus, flowers during the daytime, and therefore may be considered to be significant in this connection. Although I was obliged to leave the Kartabo Station before this species flowered, Miss Anna H. Taylor and Dr. and Mrs. Alfred Emerson very kindly consented to watch for the flowering season, and were able to make a number of important observations. I have already referred to the fact that "a humming bird, believed to be the species *Topaza pella* or the long-tailed, crimson Topaz, visited a flower in bloom and sucked nectar from the nectary." However, the bird did not approach the inflorescence from *below*, but "hovered from the *top*, dipping its slightly curved or straight bill of medium length into the nectary for a short distance." In *M. purpurea*, as in *M. cuyuniensis*, dehiscence occurs within the calyptriform corolla, and at the time when the capsule falls the stigma is already coated with a thick layer of pollen. Furthermore, the stamens show a pronounced tendency to wither, and to drop off with the deciduous capsules. Dr. Emerson states:

> The caps split at the base and work slowly off. When they fall, they often carry a large number of stamens with them. In a few cases, when the caps were about halfway off, we could see that many of the stamens had withered at the base and would very likely fall with the cap. The pistil is covered with pollen at the time that the cap falls.

Evidently the day-flowering *M. purpurea* is self-pollinated like the night-flowering *M. cuyuniensis*.

Discussion

Such facts as these raise the question whether the Marcgraviaceae are protandrous and whether their curious nectariferous appendages are adaptations to insure cross-pollination. Although the inflorescences of certain Marcgraviaceae are known to be visited at times by insects and birds, there is no reliable evidence to indicate that these animals actually are concerned in the pollination of the flowers. Statements to the effect that certain species are entomophilous or ornithophilous are based upon purely teleological inferences, rather than upon detailed field observations and carefully planned experimental controls. Furthermore, the prevailing view that the Marcgraviaceae are protandrous may be traced back to Delpino (1869), whose conclusions appear to have been derived, not from the study of living plants, but from the examination of herbarium material and from Martius' figures of certain species of Ruyschia and Norantea.

It is essential, therefore, that various species of Norantea, Ruyschia, Souroubea, and Marcgravia be critically studied in order to determine (1) whether any of the Marcgraviaceae are protandrous, and (2) whether the insects and birds which visit the nectaries actually are concerned in cross-pollinating the flowers.

Delpino (1874), Belt (1874), Kerner (1876), and certain of their contemporaries were of the opinion that extra-floral nectaries are adaptations for attracting insects or other animals. The theories of these investigators have been severely criticized, if not actually demolished, by Rettig (1904), von Üxküll-Güldenbrandt (1907), von Ihering (1907), and others, and it must be admitted that biologists are still as ignorant as they were in the days of Linné concerning the true function of the extra-floral nectaries and the so-called food-bodies of plants. Therefore, it is to be emphasized, in conclusion, that the hypophyllous glands and nectariferous appendages of the Marcgraviaceae, and extra-floral nectaries and "food-bodies" in general, deserve to be studied intensively along physiological lines.

Conclusions

1. Although the inflorescences of Marcgraviaceae are visited at times by insects and birds, there is no reliable evidence to indicate that these animals actually are concerned in the pollination of the flowers.

2. The highly specialized inflorescences of *Marcgravia umbellata* L., *M. cuyuniensis* spec. nov., *M. purpurea* spec. nov., and of similar species do not appear to be efficient mechanisms for insuring cross-pollination by humming birds. The pedicels and nectaries are so arranged that birds tend to approach the inflorescences from *above* and, therefore, do not become coated with pollen which subsequently is rubbed off on the pistils of other flowers.

3. The flowers of the only two species of Marcgravia, *M. cuyuniensis* and *M. purpurea*, which have been studied in detail in the field, appear to be self-fertile or autogamous, instead of being protandrous and cross-pollinated by birds.

ACKNOWLEDGMENTS

I am indebted to the Committee on Grants for Research of the American Association for the Advancement of Science for a grant of $500 which enabled me to undertake this and a number of other investigations. I wish to thank William Beebe, Director of the Tropical Station of the New York Zoölogical Society, for the privilege of visiting the Kartabo Laboratory, and for his constant endeavor to facilitate my investigations. Miss Anna H. Taylor, Dr. and Mrs. Alfred Emerson, and Mr. John Tee-Van very kindly contributed a number of observations, drawings, and photographs which were much appreciated. My colleague, Professor B. L. Robinson, offered a number of helpful suggestions in phases of the problem relating to his specialty.

LITERATURE CITED

Belt, T. 1874. The naturalist in Nicaragua. Pp. xvi + 403. London.

Bryant, E. M. 1905. The pollination of exotic flowers. Nature **71**: 249.

Delpino, F. 1869. Rivista monografica della famiglia delle Marcgraviaceae. Nuov. Giorn. Bot. Ital. **1**: 257–290.

——. 1874. Ulteriori osservazioni e considerazioni sulla dicogamia nel regno vegetale. Atti. Soc. Ital. Sci. Nat. Milano **16**: 151–349.

Ihering, H. von. 1907. Die Cecropien und ihre Schutzameisen. Bot. Jahrb. (Engler) **39**: 666–714.

Jussieu, A. L. de. 1809. Sur une nouvelle espèce de Marcgravia, et sur les affinités botaniques de ce genre. Ann. Mus. Hist. Nat. **14**: 397–411.

Kerner von Marilaun, A. 1876. Die Schutzmittel der Blüten gegen unberufene Gäste. Festschr. Zool.-Bot. Ges. Wien 1876: 187–261.

Müller, H. 1873. Befruchtung der Blumen durch Insecten und die gegenseitigen Anpassungen beider. Pp. viii + 478. Leipzig.

Planchon, J. É., and Triana, J. 1863. Sur les bractées des Marcgraviées. Mem. Soc. Sci. Nat. Cherbourg **9**: 68–88.

Rettig, E. 1904. Ameisenpflanzen—Pflanzenameisen. Beih. Bot. Centralbl. **17** : 89–122.

Schimper, A. F. W. 1888. Botanische Mittheilungen aus den Tropen I. Die Wechselbeziehungen zwischen Pflanzen-Ameisen im tropischen Amerika. Pp. 59–92. Jena.

——. 1898. Pflanzengeographie, auf physiologischer Grundlage. Pp. xvii + 876. Jena.

Seemann, B. 1870. A new pitcher-plant from Central America. Jour. Bot. (London) **8**: 245–246.

Szyszylowicz, I. von. 1895. Marcgraviaceae, in Engler and Prantl's "Die natürlichen Pflanzenfamilien" III, **6**: 157–164.

Üxküll-Güldenbrandt, M. Niewenhuis von. 1907. Extraflorale Zuckerausscheidungen und Ameisenschutz. Ann. Jard. Bot. Buitenzorg **6**: 195–327.

Wittmack, L. 1878. Marcgraviaceae, in Martius' "Flora Brasiliensis" XII, **1**: 214–258.

DESCRIPTION OF PLATES

PLATE XXIII

FIG. 1. *Marcgravia purpurea* spec. nov. Foliage and immature inflorescences. Photograph by John Tee-Van. × 5/22.

FIG. 2. *M. cuyuniensis* spec. nov. Section of leaf, showing outlet of hypophyllous gland. × 30.

FIG. 3. *M. purpurea*. General habit of growth. Photograph by John Tee-Van. × 1/30.

FIG. 4. *M. cuyuniensis.* General habit of growth and bagged inflorescences. Photograph by John Tee-Van. × 1/26.

PLATE XXIV

FIG. 5. Same. Section of seed showing embryo. × 42.

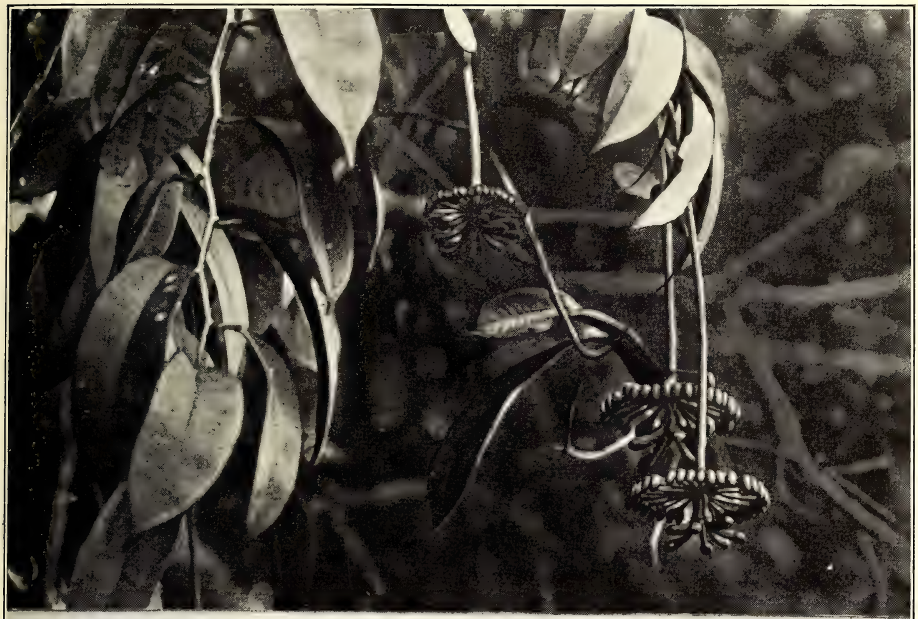
FIG. 6. Same. Transverse section of nectariferous appendage, showing two fibrovascular systems. × 30.

FIG. 7. *M. purpurea.* Transverse section of flower bud. × 8.

FIG. 8. *M. cuyuniensis.* Section of fruit, showing seeds (light) and abnormal ovules (dark). × 26.

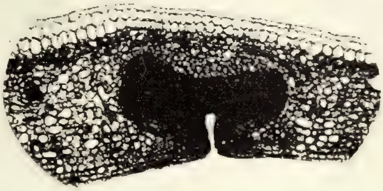
FIG. 9. Same. Transverse section of nectariferous appendage, showing outlets from glandular tissue and rudimentary flower bud. × 9.

FIG. 10. Same. Transverse section of flower bud, showing parietal placentation. × 6.

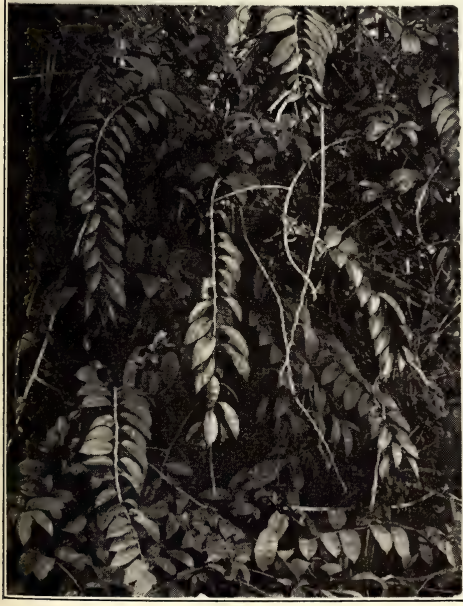


1

2

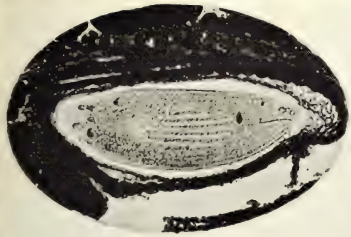


3

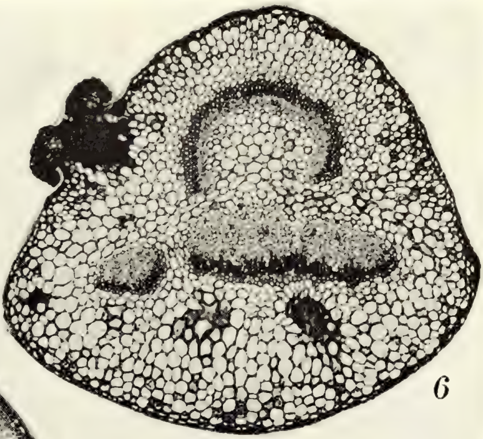


4

BAILEY: POLLINATION OF MARCGRAVIA

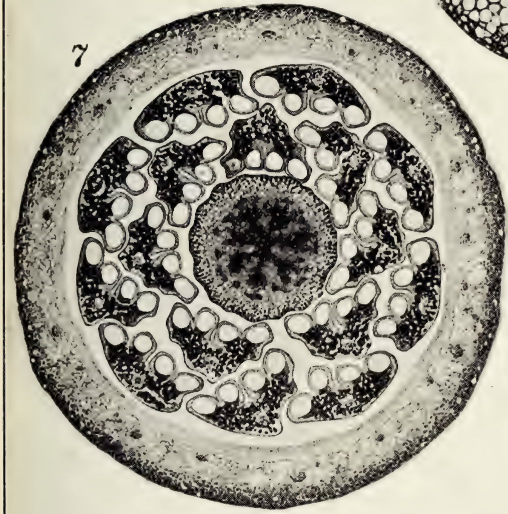


5

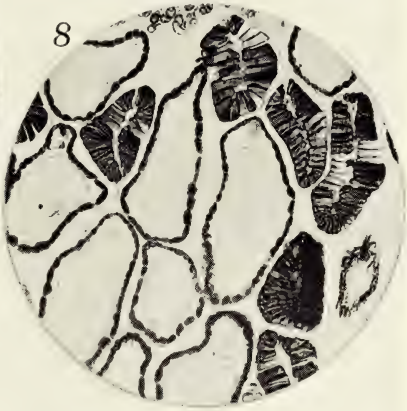


6

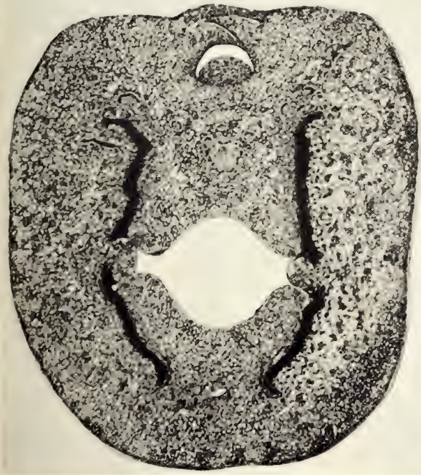
7



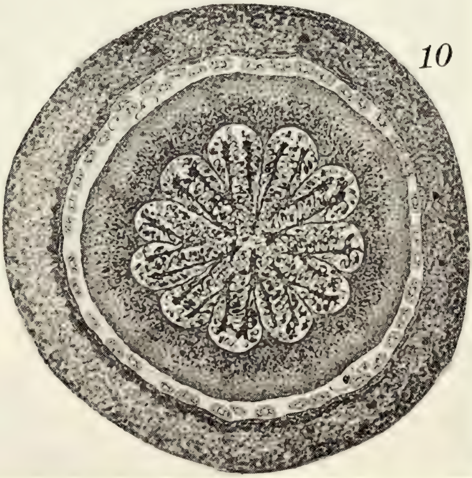
8



9



10



Bailey: Pollination of Marcgravia

ENVIRONMENTAL TEMPERATURES OF FUNGI IN NATURE

Neil E. Stevens

(Received for publication December 12, 1921)

The relation between environmental temperatures and the development of certain fungi—particularly plant parasites—has been the subject of recent study by several investigators.[1] The results of these studies, while highly suggestive, have, of necessity, been somewhat inconclusive because the only material available has consisted of the temperature relations of certain fungi as determined by their behavior in pure culture and of climatic temperatures, *i.e.*, the temperature of the air in shade. The unsatisfactory nature of comparisons made on this basis has been frankly recognized by most of the investigators. Fawcett, for example, remarks in his introduction (p. 184):

> Most organisms (aside from warm-blooded animals) are never exposed, in nature, to maintained temperature for any considerable period of time; their temperature environment is practically always in a state of flux. From this it follows that a knowledge of the relation holding between maintained temperatures and vital processes, no matter how thorough such knowledge may be, can not be expected to be a complete basis for an interpretation of physiological processes going on under natural conditions.

The study of the relation of fungi to their environment is, however, still more complicated by the fact that, as the present notes show, the plant parts living or dead upon which many fungi grow are often, when exposed to the sun, at a temperature much above that of the air. The temperature of these plant parts, moreover, apparently fluctuates under certain conditions much more rapidly than that of the air in the shade.

That the twigs of living peach trees when exposed to the sun often reach a temperature well above that of the air was pointed out over twenty years ago by Whitten.[2] His observations, however, were made chiefly in winter, and the greatest difference he records is 8° C. (air 2.7° C; twig 10.7° C.).

In a series of observations made incidentally during the summer of 1921, much greater differences between the temperature of the air and that of twigs exposed to the sun were frequently observed by the writer. The temperature readings here recorded were all made with mercury thermometers especially made for the work, having cylindrical bulbs 2 mm.

---

[1] Most of these papers are cited in Fawcett, H. S. The temperature relations of growth in certain parasitic fungi. Univ. Cal. Pub. Agr. Sci. **4**: 183–232. 1921.

[2] Whitten, J. C. Das Verhältnis der Farbe zur Tötung von Pfirsichknospen durch Winterfrost. Pp. 1–34. Halle, 1902 (and earlier publications).

in diameter which could readily be thrust into decayed or succulent stems, or between the bark and the wood of firmer stems. These observations may so readily be duplicated that no attempt is made to report them in their entirety, as a few examples will serve as representative of hundreds made on various hosts. They show, in brief, that at night or in the shade the temperature of twigs and small branches approximates that of the air, whereas in the sunlight their temperature is generally above, sometimes as much as 20° C. above that of the air. For example, dead twigs of the cultivated currant (*Ribes* sp.) bearing numerous stromata of the fungus described by Grossenbacher and Duggar[3] as *Botryosphaeria ribis* showed on a clear afternoon the temperatures recorded in table 1.

TABLE 1. *Temperature in Degrees Centigrade of Dead Twigs of the Cultivated Currant Lying on the Ground, North Carver, Mass., May 26, 1921*

Time P.M.	Air in Shade	Bulb of Thermometer Under Bark	Bulb of Thermometer in Center of Twig
1:30	17	33 in sun	27 in sun
2:00	17	36 in sun	29 in sun
2:30	17.2	37 in sun	28.8 in sun
3:00	17.4	36 in sun	27 in sun
3:30	18	18 in shade	18 * in shade
4:00	18.4	18.4 in shade	
4:30	18	17.6 in shade	

* This twig, put back in the sun, rose to 27 in 5 minutes.

This example is by no means unique, since similar results were obtained with currant twigs in other localities, as well as with other dark-colored twigs such as those of black cherry (*Prunus serotina* Ehrh.) and black birch (*Betula lenta* L.). The greatest difference between the temperature of the air and that of a currant twig infected with *Botryosphaeria ribis* yet recorded by the writer was on Overlook Mountain, Woodstock, N. Y., May 13, 1921, when under continuous observation of fifteen minutes (2:00 to 2:15 P.M.) such a currant twig showed a temperature of 37° C. while the temperature of the air in the shade remained at 15.6° C. As might be expected, dark-colored twigs tend to show somewhat higher temperatures in direct sunlight than lighter-colored twigs, as may be demonstrated by exposing small branches of white, yellow, and black birch side by side. That the high temperature of the currant twigs shown in table 1 was due to the heat of the sun is readily shown by the fact that as soon as the shadow of the bank on which they were exposed reached them, their temperature rapidly fell to near that of the air. The same relation is even more clearly shown by the record of temperatures of two blackberry [*Rubus* (probably) *allegheniensis* Porter] canes given in table 2. These canes were near a patch infected with orange leaf rust, so the data given indicate to some extent the temperature conditions to which the fungus was exposed on that day.

---

[3] Grossenbacher, J. G., and Duggar, B. M. A contribution to the life-history, parasitism, and biology of *Botryosphaeria ribis*. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bull. 18. 1911.

TABLE 2. *Temperatures in Degrees Centigrade of Blackberry Canes about 1 cm. in Diameter. Thermometer Bulbs placed in the Pith. Livermore, Maine, June 24, 1921. A Day with Drifting Cumulus Clouds*

Time A.M.	Air in Shade	Live Cane	Dead Cane
9:00	24	24 Shade	24 Shade; no sun on plant yet
9:30	24.5	30 Sun	30 Sun
9:45	24	29 Shade	27 Shade
10:00	24	30 Sun	30 Sun
10:50	24	31 Sun	31 Sun
11:00	25	32 Sun	32 Sun
P.M.			
12:30	24.5	31 Shade	31 Shade (for a few minutes)
1:00	25.5	32 Sun	31 Sun
1:30	26	28 This cane shaded by its own leaves	31 Sun
1:35	26	27 This cane shaded by its own leaves	28 Shade
1:40	26	29 This cane shaded by its own leaves	32 Sun
2:00	26	29 This cane shaded by its own leaves	33 Sun
2:30	27	28 This cane shaded by its own leaves	35 Sun
3:00	26	27 This cane shaded by its own leaves	33 Sun
3:30	25	26 This cane shaded by its own leaves	31 Sun
4:00	24.5	25 This cane shaded by its own leaves	28 Sun
4:30	24	24 This cane shaded by its own leaves	27 Sun
6:30	20.5	19.5 Shade	20 Shade
6:45	20	19 Shade	20 Shade
7:00	19	18 Shade	19 Shade

Although the figures published in these notes refer only to twigs and branches, there is abundant evidence that a similar relation holds in the case of other aërial plant parts. Data showing that the temperature of various small fruits in sunlight is usually above that of the air were published in 1918.[4] Individual strawberries (*Fragaria* sp.), for example, were often found to be 10° C. or more above the temperature of the air. These observations agree with those of Müntz[5] (p. 223) on grapes. Müntz found that the temperature of grapes in the morning, before they were exposed to the sun, was only about one degree above that of the air, whereas in the early afternoon red grapes exposed to direct sunlight had, on the day of his observations, a temperature of 37° C. and white grapes a temperature of 34° C. while the temperature of the air in the shade was only 24° C. He found, moreover, that grapes with a dull surface reached a slightly higher temperature when exposed to the sun than those with a bright surface. The importance of these high temperatures in the growth of the numerous fungi found on grapes and strawberries is obvious.

---

[4] Stevens, N. E., and Wilcox, R. B. C. Temperatures of small fruits when picked. Plant World **21**: 176–183. 1918.

[5] Müntz, M. A. Recherches expérimentales sur la culture et l'exploitation des vignes. Ann. Sci. Agron. Franc. Étrang. II, 1^1: 1–272. 1895.

Dufrenoy[6] (p. 16) found that the temperature of leaves of Prunus affected by *Polystigma rubrum* when exposed to sunlight showed a temperature from 8° C. to 11° C. above that of the air. The temperature of the air at the time of the observation was 20° C. while that of the leaves varied with their color from 28.5° C. to 31° C.

The temperature of plant parts underground must fluctuate less rapidly than that of the parts exposed to direct sunlight in the air. Table 3 shows, however, that a fungus like orange leaf rust on Rubus, which is so frequently found on railroad embankments and other sandy, exposed places, may often be subjected, even when underground, to a temperature well above that of the air. The difference in the temperature of the various plants referred to in table 3 was clearly due to the difference in exposure and slope.

TABLE 3. *Temperature in Degrees Centigrade of Soil immediately about the Bases of Dewberry Plants (Rubus villosus Ait.), Affected with Orange Rust, in Typical Location on Side of Gravelly Bank with Eastern Exposure, North Carver, Mass., May 20, 1921*

Time A.M.	Air in Shade	Plant 1	Plant 2	Plant 3	Plant 4
8:00	14	17	18	17.5	18
8:30	15	18.5	30	19	20
9:00	17	20	21	20	21
P.M.					
1:00	23	22	24	25	28
3:00	23	22	22	24	26

Similarly, the weather and soil conditions recorded by Shantz and Piemeisel[7] in connection with their studies of the growth and fructification of *Agaricus tabularis* Peck and *Calvatia cyathiformis* Bosc. at Akron, Colorado, show that the mean temperature of the soil, in which the mycelia of these fairy-ring fungi were growing, was usually different from the mean temperature of the air. In some cases the difference amounted to as much as 13 or 14 degrees F. (pp. 210, 211).

---

[6] Dufrenoy, M. J. Les conditions écologique du développement des champignons parasites. Bull. Soc. Mycol. France **34**: 8–26. 1918.

[7] Shantz, H. L., and Piemeisel, R. L. Fungus fairy rings in eastern Colorado and their effect on vegetation. Jour. Agr. Res. **11**: 191–245. 1917.

That the fluctuations in the temperature of twigs exposed to varying conditions of sunlight and shade may be rapid and of considerable extent is evident from parts of tables 1 and 2. The figures there given, however, by no means represent extreme conditions. The most extreme fluctuations yet observed have been those of small dead twigs lying on the ground in full sunlight on days when the sun in an otherwise clear sky is from time to time obscured by drifting clouds. Naturally, when the clouds are not too large, are drifting at a considerable rate of speed, and are separated by fairly wide, clear spaces, the fluctuations in temperature are most rapid and have the longest range. Under such conditions the temperatures given in table 4 were recorded. Even more rapid fluctuations in temperature might be expected on very small twigs, and in measuring the surface temperatures of leaves Mrs. Shreve[8] has noted temperature changes of from one to three degrees C. within from 20 to 60 seconds. With a moderately strong wind blowing, the change amounted to five degrees in 30 seconds.

TABLE 4. *Temperatures in Degrees Centigrade of Birch Twigs on Overlook Mountain, Woodstock, N. Y., May 13, 1921, during a Period of Alternating Sunlight and Shade due to Drifting Clouds*

9:15 to 9:30 A.M.	9:40 to 10:00 A.M.	
Air temperature in the shade during this time varied from 10.5° C. to 13° C.	Air temperature in the shade during this time varied from 10° C. to 12° C.	
Black Birch (*Betula lenta* L.)	Yellow Birch (*Betula lutea* Michx.), Very Thin Bark	White Birch (*Betula alba* L.), Thicker Bark
22 Shade	36 Sun	24 Sun
30 Shade	27 Shade	17 Shade
19 Shade	33 Sun	20 Sun
27 Sun	29 Partial shade	18 Partial shade
17 Shade	35 Sun	23 Sun
30 Sun		

That the conditions under which the data recorded in table 4 were taken are extremely favorable for rapid fluctuation in temperature is shown by numerous unpublished observations made by Dr. H. L. Shantz on solar radiation at Akron, Colorado. Briefly summarized, his observations show, as indicated by figure 1, that the amount of heat received by a given area in intervals of sunlight between cumulus clouds is greater in calories than the same area would receive if there were no clouds in the sky. The explanation of this condition offered by Dr. Shantz is that the amount of heat received in the clear intervals between clouds is augmented by reflection from the surfaces of the clouds themselves.

---

[8] Shreve, Edith B. Apparatus for determining the temperature of leaves. Yearbook Carnegie Inst. Wash. **17**: 80–81. 1918.

What significance these rapid fluctuations in temperature may have on the physiology of the fungi growing on or in dead twigs, the writer does not attempt to say, but they must have some bearing on the rate of growth and fructification of these organisms. It is evident also that the temperature environment of those fungi which grow on leaves and on twigs of deciduous trees must differ widely from that usually supplied them when grown in pure culture in heated laboratories.

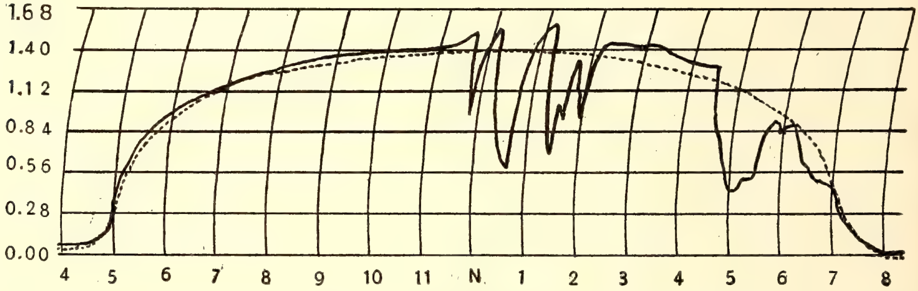


FIG. 1. Total radiation, in gram calories per minute per square centimeter of surface, at Akron, Colorado. Dotted line, July 9, 1914, a clear sky. Solid line, July 11, 1914, a day with drifting clouds.

SUMMARY

The data presented in the present paper indicate that many plant parts affected by fungi often show, when exposed to the sun, a temperature markedly above that of the air.

They also indicate that the fungi are sometimes subjected to fluctuations of temperature more rapid and extreme than the fluctuations in the temperature of the air in the shade.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

THE REDUCTION DIVISIONS IN THE POLLEN MOTHER CELLS OF OENOTHERA FRANCISCANA

Ralph E. Cleland

(Received for publication December 24, 1921)

Introduction

Investigation of the Oenotheras has reached such a stage that it is essential, if any progress is to be made in the study of the group, to obtain species concerning the purity of which there can be no question. Of all the species so far studied, *Oe. franciscana* seems to give the most promise of being undoubtedly pure. This is a far western species, which was first described by H. H. Bartlett in 1914. His description is based on material grown at Washington for the first time in 1910, from seed collected at Carmel Beach, California, in 1905. Seed from this strain was sent to Dr. B. M. Davis in Philadelphia, and grown by him under the name of *Oe. franciscana* B., which race he has carried on in selfed line since 1913. This strain has proved to be very stable. It has never thrown any mutants, has shown a high percentage of pollen and seed fertility, and in every respect acts like a well established pure race. It thus falls into a group with *Oe. biennis* L. and *Oe. grandiflora* Ait., which have also been shown to be very constant. The cytology of *Oe. grandiflora* has been worked out by Davis (1909) and has proved exceedingly interesting as revealing a very regular nuclear behavior during the reduction process, with a definite pairing of homologous chromosomes at diakinesis and the formation of a clear equatorial plate at the heterotypic metaphase. This uniformity in development is quite in contrast with what has been found to be the case in those Oenotheras which are less stable; and it therefore becomes of interest to examine the spore mother cells of the other stable species to determine whether they also may show the same regularity of development displayed by *grandiflora.*

Material

Collections were made by the writer during the summers of 1919 and 1920 in the gardens of Dr. B. M. Davis. A variety of fixing fluids were used. Of these, one of Allen's modifications of Bouin's solution (Bouin A) gave the best results, although strong Flemming with urea and maltose added (S.F.U.M.) yielded some fairly satisfactory fixations.

Description

An attempt was made to study carefully the telophase stages of the last archesporial mitoses, with a view to determining whether a definite splitting of the chromosomes into two parallel threads takes place, as described by Digby (1919), or whether the events conform rather to accounts such as those given by Sharp for Vicia (1913) and Tradescantia (1920). It was found impossible, however, to get very definite results, as the nuclei at this stage were too small, and the chromosomes too tightly packed to allow of close observation. The following account, therefore, begins with the return of the nucleus to a resting condition after the last archesporial division.

The cells of the archesporium pass through a fairly long-continued resting stage previous to entering upon the maturation prophase. They lie usually in one or two rows and fit tightly together with no spaces between them or between the archesporium and the well-differentiated tapetum. The nucleus is comparatively large (10–11 μ), very nearly spherical, and possesses a prominent nuclear membrane (Pl. XXV, figs. 1, 2). Within it may be found one or more nucleoli. Ordinarily but one of these attains to any size. It is spherical, and lies toward the center of the nucleus. Within it may often be seen one or more hyaline circular areas, resembling vacuoles. These may contain, or occasionally give place to, angular, crystal-like bodies. The other nucleoli when present are usually smaller and are stationed here and there throughout the nucleus. All of these bodies are in intimate contact with the linin network.

Scattered evenly throughout the nucleus is a reticulum of very delicate threads. Some of the threads are very thin and free from granules. Others are dotted over more or less unevenly with chromatin particles. Wherever threads join, chromatin accumulations varying in size from tiny dots to fairly large masses are to be found. These may be irregular and shapeless, or rounded and indistinguishable, perhaps not different, from small nucleoli. In well fixed material the network closely envelops the nucleolus, to which it is connected in various places. There is, therefore, no space around the nucleolus. Wherever the fixation is faulty, however, a clear space is observed, due to shrinkage of the reticulum. During the resting stage there is little if any indication of parallelism of the threads. For the most part they are single and are uniformly distributed throughout the nucleus.

The resting condition lasts for quite a time, long enough for the anther practically to double in length. Throughout this period all trace of the individual chromosomes is lost—they have been resolved completely into a network of threads.

Although there is little or no sign of parallelism of threads during the resting stage, there begin to appear occasional instances of such an approximation as the nucleus begins to draw near to the time when it will enter upon the heterotypic prophase (fig. 3). By no means all the threads become thus arranged. Most of them remain single. Moreover, the parallel threads do not come together very closely, and there is not much assurance at this stage that a fusion of the approximating threads will result. The condition is sufficiently different, however, from the typical resting condition to be noticeable and to indicate that the nucleus is approaching prophase.

The Heterotypic Prophase

The series of events which follows may be briefly defined as a process by which a small-meshed reticulum composed of many thin, often more or less unevenly granular threads becomes transformed into a large-meshed reticulum composed of fairly thick and uniform threads—a network of such large mesh, and with the threads joined to each other in so few places, that it can perhaps be better described as a spireme. The problem before us for the moment is to determine by what means this change, with the consequent reduction in the number of threads, is brought about.

The researches of Grégoire and Allen and their followers, on the one hand, and of Farmer, Digby, Mottier, and others on the other, have been in agreement as indicating that a most important element in this process is a side-by-side approximation and fusion of threads in early prophase, thus reducing the number of threads to approximately one half. For every thread there is a counterpart in the reticulum, which is so placed that the two can approach each other and fuse. The interpretations placed upon this process, however, by the two schools have been different. The former group (the parasynaptists) consider that the threads which enter into the fusion are homologous parts of two spiremes, one of which traces its ancestry back to the egg, the other to the sperm. The threads represent whole chromosomes and their fusion results in a bivalent spireme. A typical telosynaptic interpretation has recently been set forth very clearly by Miss Digby in her paper on Osmunda (1919). According to this view, the individual threads are half-chromosomes which form on "association" a univalent filament, the chromosomes descended from both sperm and egg forming a part of one spireme. Miss Digby has described in the last telophase before the heterotypic mitosis a longitudinal fission of the chromosomes into two parallel threads, which are later obscured in the resting stage. The parallelism in prophase she considers as due merely to the return of these two split parts together. According to this view, the fission in telophase is preliminary to the separation of chromosomes during anaphase of the homoeotypic mitosis. According to the Gregoirean interpretation, the two parts which fuse in prophase will separate during anaphase of the heterotypic mitosis.

While in general the method of development in *Oenothera franciscana* is telosynaptic, we shall nevertheless see that there is no evidence of any very general parallelism and side-by-side fusion of threads in early prophase such as has been observed in Osmunda. It is true that some parallelism is apparent at the very beginning of prophase, as I have already pointed out, and without doubt a certain part of the condensation of the network is effected through the agency of parallel fusions, but by far the greatest amount of it is brought about in other ways.

As the nucleus advances into prophase, the amount of parallelism does not increase materially, but the reticulum loses the even and uniform appearance that has been so characteristic of it (fig. 4). The meshes begin to show a marked difference in size, the threads are no longer so evenly distributed, and are becoming quite unequal in diameter. The difference in size of the meshes seems to be due mainly to the fact that some of the threads are contracting and becoming thereby shorter and thicker. This process serves to bring threads which have been lying far apart nearer together, so that they occasionally parallel one another quite closely. It also helps to bring about a variation in the diameter of the threads. Those which contract will become thicker thereby. Furthermore, contraction on the part of some threads will result in the stretching and attenuation of others. Another process seems to be going on at the same time, however, which is doing much to make some threads thicker than others. There are indications of a flow of material from one thread to another, or from one part of a thread to another part, resulting in a marked thickening of some parts and the absorption and disappearance of others. One may observe everywhere threads whose contents seem to have passed to adjacent knots or threads, causing these to enlarge, while the former have themselves become very thin and are either on the point of breaking or have already broken (figs. 4–7). It is evident that certain threads are being sacrificed, and that the material of which they are composed is being transferred to the threads which are to survive, or is being temporarily stored in the "knots," formed where the threads join. A process of this kind, involving a certain amount of contraction, will necessarily result in occasional parallelisms. They are not sufficiently numerous in this material, however, to warrant the belief that they are of any far-reaching significance. They are what might be termed "chance" parallelisms.

Synizesis. The condition of synizesis is apparently brought about by the continued contraction of the threads of the network. There is so much pull exercised upon the peripheral threads because of this shrinkage that they become torn away from the nuclear membrane on one side, and the contracting mass of threads shrinks into a bundle against the opposite side of the nucleus. Either before this breaking away occurs or while the bundle is being formed, the nucleolus leaves its central position and comes to lie against the membrane in the midst of the reticulum (figs. 4, 5, 8, 9). Here it becomes somewhat flattened and cushion-shaped.

The position of the synizetic knot is of interest. One cannot help being struck by the fact that during synizesis the threadwork lies, in the vast majority of cases, on that side of the nucleus which is nearest the periphery of the cell. Just what significance should be attached to this fact I am not prepared to say. The question naturally arises, however, as to whether such a position might not be due to strong exosmotic currents which would pull the reticulum toward that side of the nucleus first affected by the fixing fluid. If it be true that the position of the knot is due to the action of the fixing fluid, one might perhaps be justified in harboring slight suspicions as to the cause of the shrinkage itself.

In early synizesis the whole reticulum is so massed together and the individual threads are so fine that it is almost impossible to distinguish anything in detail. The only way really to observe what is taking place is to study thin tangential sections of the knot. We are in this way enabled to see that the same process which was going on just previous to synizesis, by which certain threads were being absorbed while others were becoming enlarged, is still in progress. The network is at first much like that which entered synizesis, except that the meshes have become smaller by contraction, and the whole has thus become much more compact (figs. 5–7). The threads, however, rapidly become less and less uniform in appearance, as certain of them give up their contents to others (fig. 6). As the result of this process, some of the threads begin to stand out more and more prominently, so that, as later and later stages are reached, it becomes apparent to one examining the knot as a whole that it is reticulate in nature. One can distinguish threads running here and there throughout the densely packed mass. Tangential sections of late stages show that the finer threads are fast disappearing and that those which remain are becoming not only more prominent, but on the whole more uniform in diameter and less granular (figs. 8–10).

Certain of the threads in the center of the reticulum, and often in close juxtaposition with the nucleolus, may in time become especially prominent (Pl. XXV, figs. 9–12; Pl. XXVI, fig. 13). They swell greatly and evidently come to contain a great deal of chromatin material. A whole section of the network may become thus involved, with the result that a large, more or less shapeless body is formed in the middle of the reticulum. The nearness of this mass to the nucleolus suggests that the latter may be emptying itself of its chromatin contents, the rate of emptying being greater than the rate at which it can flow out into the various parts of the reticulum. These aggregations disappear more or less completely by the beginning of the open spireme stage (fig. 14).

During late synizesis the spireme becomes continually clearer and more uniform, as the threads which are not to survive disappear and their material, and that from the nucleolus, becomes more evenly spread over the thread system (Pl. XXV, figs. 8–10). While the surviving threads are much thicker than those of the pre-synizetic reticulum, they do not continue to thicken indefinitely. Instead, they lengthen considerably to make room for the additional chromatin material. This lengthening process may very well be the cause of the unfolding of the tangled knot. A reticulum of such long threads cannot remain in such a small space unless the threads are very sharply and frequently bent and twisted. There is a tendency to resist this excess of bending, to straighten out, and hence the unfolding.

The nucleolus, which at the beginning of synizesis moved over against the nuclear membrane, becomes by mid-synizesis not only flattened but much spread out and quite often assumes the shape of a horseshoe or doughnut. That it is comparatively empty is shown by the staining and by the fact that an endonucleolus now stands out very clearly within it as a black-staining, spherical little body. This body makes its first appearance in early synizesis and remains a constant feature of the nucleolus until the time of the disappearance of the latter during diakinesis (fig. 11).

Synizesis is, then, the time during which transition is made from a thin-threaded reticulum with scattered chromatin particles to a thick-threaded spireme. This transition does not appear to be brought about by the parallel fusion of two separate thread systems, but by a process which consists largely of the contraction of certain threads and the absorption of their contents into the body of other threads, a process which involves only occasional parallelisms. It seems, also, that the total amount of chromatin on the threads is greatly augmented during this process, and it is not unlikely that the extra chromatin has been brought out of the nucleoli and distributed along the network.

The Open Spireme. The unfolding of the synizetic knot is gradual. It is accompanied by little further thickening of the spireme. The large irregular accumulations of chromatin which were formed in the center of the knot during synizesis are dwindling, though the threads at the center are usually found to be somewhat thicker than the rest of the network through the whole of the open spireme stage (Pl. XXV, fig. 12; Pl. XXVI, figs. 13–15).

Judging from the frequency of its appearance, the open spireme stage is one of fairly long duration. The threads are long and evenly distributed through the nucleus (fig. 14). They frequently show a distinct and regular beading. It is often possible to trace a single thread for a long distance as it winds and twists through the nucleus. Here and there it joins with other threads, the whole system constituting not a single coiled or twisted thread but a loose reticulum. Ordinarily it is not possible to locate any threads with free ends.

During the open spireme stage the threads are characteristically single and unsplit. Wherever they are prominently beaded, the beads are found to be arranged in a single row, stretching quite across the thread and showing no sign of division. Once in a while one may find in late synizesis a lingering trace of parallelism, but in the open spireme we rarely have anything to indicate that the thread might be by nature double. Occasionally two parts of the spireme may lie rather close together, and the parallelism may strike one at first, but it is quickly evident that this is merely the chance lying together of two whole threads which, when traced a little distance, are seen to have no immediate connection with one another. During this stage, also, the nucleolus begins to round off and leaves the nuclear membrane, where it has been lying through synizesis. It usually takes the stain very lightly so that the endonucleus shows clearly within. It is an interesting fact that the latter body seems quite often to be clearly connected with a part of the spireme (fig. 16).

During synizesis and the open spireme stage, the pollen mother cells, which during the resting period were packed closely together, begin to round off and to separate somewhat from each other, though they do not become entirely detached. The anther and the loculus in which they are placed become considerably enlarged during this time, but the cells themselves grow only slightly. The nuclei also do not grow appreciably, measurements during the open spireme and second contraction stages showing an average diameter of 12–13 μ, as compared with 10–11 μ in the previous resting phase.

Second Contraction. After a time the nucleus begins to pass out of the open spireme stage and to enter upon the stage known as "second contraction." The spireme, which has hitherto been rather evenly distributed throughout the nucleus, begins to thicken and shorten, the central portion condensing very rapidly, while the peripheral part becomes thrown into loops which radiate more or less clearly from the central region (fig. 15). The threads in the center of the nucleus become thicker and thicker, until they gradually lose their thread-like form and become great, irregular, swollen masses which, as they increase in size, come to lie against one another so closely that the individual parts become lost and it is usually impossible to tell whether they have amalgamated or not (figs. 16–18). This process results from the passage of material into the region from the rest of the spireme, a process which results also in a shortening of all the threads of the reticulum.

As condensation goes on and this central mass becomes larger and larger, all the threads of the reticulum shorten, and it becomes more clearly evident that the threads which pass out toward the periphery are arranged in loops (figs. 15–18). Even in early stages of the second contraction it can be seen that the threads are forming loops which run out to the periphery and which are attached to each other in places (fig. 15). At first they are so long that they must bend in various ways, but as the threads shorten, the loops become smaller and smaller, so that at last they can stretch their full length without touching the periphery. The nucleus then begins to present a very striking figure, the chromatin mass resembling a large, irregular central hub from which the loops radiate out like spokes in a wheel (fig. 19). As the loops become shorter, their two sides may begin to approximate until in some cases they actually lie against one another (figs. 19, 20). They do not twist around one another, however, to form the characteristic condition known as strepsinema. When the climax of condensation has been reached, the loops are sometimes so short that they are barely visible beyond the edge of the central mass (fig. 21). In practically no case, however, are they entirely lost sight of. Throughout the whole of this period at least some of the loops are in evidence, and it seems certain that they form the bivalent chromosomes which are soon to emerge from the contracted mass.

The nucleolus is usually not involved in this condensation. During the second contraction it ordinarily retreats again to the nuclear membrane, where it begins to flatten out once more. It still shows as clearly as ever the black-staining endonucleolus (figs. 18–20, 23).

It is important to notice that there is almost no indication of the occurrence of any kind of split in the spireme during this process of contraction. In only a very few instances have I found any trace of it, and these were in poorly fixed cells. It is possible that about or before this time the spireme begins to divide lengthwise in preparation for the homoeotypic division, and that this split occasionally shows. If the spireme were bivalent in nature, however, we ought to find at this stage, when the chromosomes are preparing to separate from each other, that the spireme is everywhere splitting into two threads. I have been unable to find evidence that such a splitting occurs. The thread is constantly single.

As the loops become shorter and thicker, the first indications of the approaching cross-segmentation of the spireme make their appearance in the form of constrictions (figs. 16, 19, 21). A constriction may be found at the distal end of the loop, in which case each side of the loop represents a chromosome. In some cases a single chromosome makes up the whole of the distal portion, its ends being found part way down the sides (fig. 19). Such a loop might be made up of two, or of more than two chromosomes. The distal chromosome might be attached at both ends to the same chromosome, the middle part of which is hidden in the central mass, or the ends might be in contact with different chromosomes. We shall see shortly that the latter condition is probably always found in the case of one of the loops.

That the chromosomes are placed end to end in the spireme which makes up these loops is indicated by the fact that the individual loops may be traced entirely through the second contraction stage without showing any longitudinal splitting, emerging at the end of the period, each as a pair of chromosomes which are attached to each other by their ends. There is little doubt in my mind but that the segmentation which appears in the loops as these are contracting represents the separation of univalent chromosomes and not of bivalent ones.

Diakinesis. Up to this point we have been dealing with the spireme as a whole. During the earlier stages of prophase the thread system has been too long and complexly woven, and in the second contraction the massing of the material in the center has rendered conditions too obscure, to permit a study of the individual chromosomes to be made. From now on, however, it becomes increasingly possible to make such a study, and to determine what relation the individual chromosomes bear to each other and whether or not this relation is uniform. The conditions that we find are very interesting.

When the second contraction knot begins finally to loosen, it does so in a definite way. It lengthens out, and from a more or less rounded, spherical mass assumes a roughly cylindrical form (figs. 22, 23). As it grows longer, the individual bivalent chromosomes which compose it begin to show more clearly (fig. 23), and, despite the fact that the figure is still obscurely massed in the center, their appearance suggests a definite arrangement. From one end of the figure it is common to find a single loop or ring protruding, and from the other end, two loops. Various others may be seen emerging from the sides of the central cylindrical portion. At this time the exact arrangement of the chromosomes is not clear, but the general cylindrical shape of the whole, and the uniform placing of such loops as can be seen clearly, indicate that they do have a definite positional relationship to each other.

As the figure opens out more and more and the nucleus passes into diakinesis, it becomes possible to make out the arrangement with greater clearness. The central part of the figure resolves itself into a closed circle, composed of four univalent chromosomes attached end to end (fig. 24). Linked to this circle at different places are rings, each of which is composed of two univalent chromosomes which are attached to each other at both ends. To certain of these rings there is linked in turn another ring made up in the same way. It is possible to count these rings in most cases, and we find that there are five of them present, which, together with the circle of four chromosomes, make a total of fourteen chromosomes, a circle of four and five pairs.

The chromosome pairs begin to separate from each other almost as soon as the second contraction knot begins to loosen, so that it has been impossible to find a case in which all the chromosomes were still occupying their original position with reference to one another, after the knot had completely loosened. Fortunately, however, the rings do not seem to separate in the same order at all times, so that it has been possible by studying many cases of linking to get quite a definite idea of the original arrangement; and in spite of the fact that I have found no case in which they have all remained together long enough to be clearly observed, I have become quite convinced that there is a definite arrangement of the chromosomes which is rarely if ever disturbed. In the first place, it is certain that the closed circle of four chromosomes is practically always present. I have found no case in which it was certainly lacking, but every good nucleus in diakinesis has displayed it beyond question; so that it seems perfectly clear that this grouping together of four chromosomes is a natural and regular proceeding. In the second place, a study of the various ways in which the bivalent rings are found linked to this circle and to each other in early diakinesis has made it seem very probable that here also we are dealing with a very definite arrangement. No more than three rings have ever been found attached to the circle of four. No more than two of these rings have been seen in a nucleus with other rings linked to them, and there have been no instances observed in which more than two rings were certainly linked together (Pl. XXVI, fig. 24; Pl. XXVII, figs. 25–27). The presence of three rings linked to the circle of four has been quite often seen, however, and the two two-linked chains are frequently in evidence. These facts taken together would seem to indicate that before the bivalent chromosomes begin to separate, there are three pairs or rings which are linked to the circle of four, and that two of these rings have linked to them in turn each another ring, making five rings in all. Judging from the frequency with which the various combinations occur, it is probable that the ring which has no other ring attached to it is most often the first one to break loose from the combination. Occasionally, however, one of the other rings will come off first.

Such a definite linking of chromosomes is especially interesting for two reasons. First, it suggests very strongly that at earlier stages also the chromosomes may have a definite position in the spireme, so that when the latter condenses to form the chromosomes, they will always find themselves linked in the same way. Such a uniformity of linking as is seen in early diakinesis can be accounted for in only one way, as I see it, and that is by assuming that when the chromosomes are yet in the spireme condition they always occupy the same relative positions, and that the individual threads of the spireme are also definitely placed, connecting with each other at the same points and interweaving in the same manner in all nuclei. Furthermore, it seems to me likely that this definite arrangement does not begin with the spireme stage, but goes back through synizesis into the resting condition. It may be that the chromosomes in somatic cells also are always arranged in the same way.

In the second place, this uniform arrangement is interesting because it furnishes a very good bit of evidence for the telosynaptic arrangement of the chromosomes and for the univalence of the spireme thread. If the homologous chromosomes were arranged side by side in the spireme, it is difficult to see how linking could occur. It could easily be brought about, however, if the chromosomes were to represent segments of the whole spireme placed end to end. The arrangement would then be brought about naturally in condensation of the spireme because of the original position of the threads which later become the chromosomes.

The bivalent rings usually separate from each other and from the circle of four after a time (fig. 28). The circle of four, however, does not break up in diakinesis, but remains until the disappearance of the nuclear membrane, and even later, being often seen during the formation of the equatorial plate and even occasionally until metaphase (fig. 29). In some cases one or more of the rings remain linked to the circle of four until the end of diakinesis, but more usually we find in late diakinesis that they have all separated, so that we have the circle of four, and five pairs of chromosomes scattered about in the nucleus. One of the most interesting features of this stage is its remarkable clarity. The chromosomes are clearly distinct from one another, often attached together only by narrow threads so that they stand quite apart. The presence of such a circle of four chromosomes has been observed before. Miss Digby (1912) has described this condition in one of the Primula hybrids. Her figures undoubtedly testify to its existence in her material, but it evidently does not show as clearly as it does in *Oenothera franciscana*.

During diakinesis the nucleolus is plastered against the nuclear membrane and is fast disappearing. At times it appears vacuolated, at others it is evenly translucent and apparently empty except for the black-staining endonucleolus which is often still prominent. The nucleolus seems to be melting. The edges are becoming hazy and ill-defined, and the structure looks as though it were being dissolved away. I have seen the endonucleolus in a few cases apparently in the act of being liberated, through the melting away of the nucleolus, and it is very probable that this little body maintains its identity for some time after the nucleolus has disappeared.

Metaphase

It is not until the nuclear membrane is on the point of breaking down that signs of spindle formation become evident. The first indication of this formation is a sudden increase in density of the cytoplasm close to the nuclear membrane. At first no fibrils are to be seen. These do not appear until the nuclear membrane begins to dissolve. Then they suddenly make their appearance on all sides, and, as the membrane melts, penetrate into the nuclear region. Within a short time, a very prominent multipolar spindle is formed, with several thick clusters of prominent fibrils. As these pass into the nucleus, and in and around the chromosomes, those of each cluster anastomose in various ways with fibrils from other bundles (fig. 29).

While the spindle is being formed and the nuclear membrane is disappearing, the chromosomes rapidly shrink, until they are less than half the size which they have presented during diakinesis. They have probably had a somewhat spongy texture, and are now being greatly compacted. During the whole of the period when the spindle is still multipolar, the circle of four remains as a rule unbroken, and even shows frequently one or more rings still linked to it. During this stage also a small but prominent black-staining little body is usually in evidence, which can hardly be other than the endonucleolus, which appears to outlast the nucleolus in most instances. There is no evidence, however, that this is a permanent structure which is being carried over into the daughter nuclei. It soon after disappears and probably suffers a dilatory but like fate with the nucleolus.

The chromosome pairs are drawn to the center of the cell as the spindle becomes bipolar, and soon become arranged in a definite plate. The circle of four soon breaks into two pairs, so that there come to be seven pairs of chromosomes lying side by side in the equatorial region. The condition at this time is generally one of great regularity. Most of the pairs are still in the form of rings, and one could not imagine a more typical picture of metaphase. The appearance is especially striking after the univalent chromosomes begin to be pulled apart, and before they have actually separated (fig. 30).

Anaphase and Telophase

The spindle fibers usually attach themselves to the middle of the chromosomes, which are V-shaped as they pass to the poles (figs. 30, 31). Occasionally, however, chromosomes are fastened by their ends to the fibers, in which case they appear rod-shaped. During anaphase the chromosome do not split preparatory to the homoeotypic mitosis. They pass entire to the poles where they form a compact little cluster, one chromosome usually occupying the center of the group with the other six lying around it (fig. 32). About this little group there develops a small vacuole-like region, the outer boundary of which becomes the nuclear membrane. At the same time a few little threads are here and there thrown across from one chromosome to another, forming slight connections.

Interkinesis

The daughter nuclei, so constituted, grow rapidly. The individual chromosomes are thus enabled to separate more and more, and tend to take a parietal position. As the nucleus increases in size they become more irregular in outline, but always remain perfectly distinct and separate, being attached to each other only in the most meager way by a few scant threads (fig. 33). Nucleoli also begin to make their appearance at this time, appearing at first as minute, faintly staining globules, which gradually grow in size, never becoming large, however, or appearing to exercise any important function (figs. 33, 34). There are usually several of them in a nucleus, and they develop in contact with the chromosomes or with the chromatin threads. The fact that several of them appear synchronously in various parts of the nucleus, and that they seem to have such an intimate connection with the chromosomes, suggests that they arise *de novo*, and are not descended from previously existing bodies such as the endonucleolus.

It is not until late interkinesis that the irregularity in the shape of the chromosomes begins to give place to a definiteness of form. Shortly before the disappearance of the nuclear membrane preparatory to the homoeotypic mitosis, it becomes clear that the chromosomes have split, and that the halves are swinging around in such a way that they cross. Oftentimes they bend over one another in a very interesting way (fig. 34), at other times they remain straight. In either event, the general appearance is that of a Maltese cross.

The Homoeotypic Division

The spindle for the homoeotypic mitosis begins to develop shortly before the nuclear membrane breaks down. Here again a very prominent multipolar spindle is formed, which later becomes bipolar. The chromosomes, which lie scattered in the cytoplasm after the disappearance of the nuclear membrane, become very short and are clearly paired. They are brought to the equatorial region (fig. 35) and are separated, and seven chromosomes pass to each pole in an entirely regular way. The two figures lie at opposite sides of the cell, with their axes in a majority of cases in the same plane, though this position is not constant (fig. 35).

The granddaughter nuclei are constituted in much the same manner as were the daughter nuclei. The seven chromosomes lie at first very close together, and become surrounded by a vacuole whose outer boundary becomes the nuclear membrane. The nuclei grow rapidly and pass quickly into a resting state in which all trace of the individual chromosomes is lost. The process of expansion of the chromosomes is very clear and can easily be followed (figs. 36–38). Each chromosome begins to lengthen out and becomes a thread, which loses in diameter as it gains in length (fig. 36). Each of these threads may attain a length equal to, or greater than, the diameter of the nucleus. As each chromosome lengthens, it can be seen that very delicate strands begin to extend out from it in every direction, and these threads, coming from the various chromosomes, meet and fuse, forming a very delicate reticulum. We have then at this stage seven prominent threads, joined together by a loose network of very delicate ones (fig. 37). The contents of the original threads then pass out in varying degree into the more delicate strands, so that in time it becomes impossible to tell where the original chromosomes lay (fig. 38). After the material is thus rather thoroughly distributed, and the nucleus has reached the resting condition, it is seen to possess a loose reticulum, dotted over with chromatin particles of various sizes, and also one to several nucleoli, which have arisen as in the daughter nuclei. It is especially to be noted that there is no indication of a splitting of the chromosomes during this period. The process seems to be one purely and simply of expansion—increase in length of the original bodies, formation of branch threads by an outflowing of linin material, and distribution of the chromatin contents along them. Whether or not this telophase may be considered as throwing any light upon the telophase problem brought up by Digby (1919) may of course be open to question.

The four nuclei come to lie equidistant from one another, so that when the new walls are formed the resultant spores are arranged in tetrahedral fashion. Between them, more or less prominent sets of spindle fibers often appear. Sometimes these are very marked, at other times there is hardly a trace of them, so that when present they are probably functionless. The walls separating the spores are not formed until after the nuclei have entered fully upon the resting stage. They are all formed at one time, appearing at first as delicate cleavage planes, which later become thicker and more prominent. If cleavage begins at the surface of the cell, it passes to the center with great rapidity. I am inclined to believe that it may be brought about almost simultaneously everywhere in the planes where the walls are to form. There is no evidence that the walls develop centrifugally, by the formation of cell plates between the nuclei. No cell plates were observed, even in cases in which the spindles were most prominent, and there can be but little doubt that the division is the result of very rapid furrowing.

Discussion

The results of this investigation are of interest from the standpoint of nuclear cytology, and also because of their bearing upon problems in Oenothera genetics. We will first take up the cytological questions involved, and follow these with a brief consideration of the genetical significance of the results.

Cytological

Telosynapsis vs. Parasynapsis. One of the first questions that naturally arises during the study of maturation divisions concerns the arrangement of the chromosomes and the nature of the spireme thread. The evidence in this case seems to lead one to a telosynaptic interpretation. During the early heterotypic prophase, there is little to lead one to suppose that the chromosomes are undergoing synapsis. For the most part, the threads are unpaired. The occasional bits of parallelism to be seen at this stage are about as much in evidence as one would ordinarily expect in the transformation of a reticulum into a spireme. They are not sufficiently numerous or sufficiently extended to warrant one in considering them evidences, either for the synapsis of whole chromosomes, or for the fusion of the halves of split chromosomes. Although I have not been able to study the somatic telophases antecedent to the heterotypic prophase, the results which Sharp (1913, 1920) has obtained from the study of Vicia and Tradescantia will probably hold perfectly well in *Oenothera franciscana* also. He has found that the alveolation of the chromosomes in telophase is too irregular to be called a splitting, and that in prophase the chromosomes are fully formed by the reverse process of condensation before a split occurs. I have come strongly to the conclusion that the spireme in the heterotypic prophase of this plant is formed by an irregular process of condensation, rather than by the approximation of two distinct threads. This conclusion not only conflicts with the idea of an early split in the chromosomes of this plant, preparatory to the homoeotypic mitosis, but also with that of the possibility of synapsis, or side-by-side approximation of whole chromosomes, taking place during this period.

During synizesis, the process can be seen to be a continuation of that described above. Little indication of parallelism of any kind can be found, and what little bit there is, is only what one might expect from chance, or from the unequal condensation of the various threads of the system. When the spireme is finally formed, all the evidence seems to support the theory that it is univalent in nature. From late synizesis to the end of the second contraction stage, it remains uniformly and evenly single. As it begins to condense and shorten, so that the long, tangled loops into which it is thrown stand out more and more clearly, the single nature becomes all the more noticeable, for it is at this period that the split, in many plants and animals, becomes especially evident, and the separated threads twist around one another forming the characteristic "strepsinema" condition. In this plant, however, there is no true strepsinema, or twisting of threads, for there is no split in the spireme. The split in preparation for the homoeotypic mitosis does not appear in this plant, except rarely, until the middle or end of interkinesis.

As the spireme becomes more and more massed in the center of the nucleus during second contraction, the loops become very short and thick, and it can be seen that the chromosomes forming the loops are attached end to end. These loops can be traced throughout the whole of the second contraction period, and it seems quite certain that the ring-shaped bivalent chromosomes which emerge from the knot are the loops which entered it. Each bivalent seems to have come, therefore, from an unsplit section of the spireme. It is true that toward the end of the second contraction period I have found a very few cases in which there seemed to be some evidence of a split. These instances were so rare, however, in comparison with the cases in which the thread seemed perfectly whole, that the only interpretation possible is that they represent a premature split (premature for this plant, though usual in most if the telosynaptic view is correct) in preparation for the homoeotypic division.

Diakinesis also presents strong evidence for the telosynaptic arrangement of chromosomes in the constant and uniform linking together of bivalent chromosomes, a phenomenon which can hardly be explained on a parasynaptic basis. The most reasonable explanation seems to be that the bivalent chromosomes represent sections of the spireme which occupied such a position in the nucleus that, when the whole system became condensed, the chromosomes found themselves linked in this very definite way. It is not likely that a chromosome could pass between two members of a bivalent pair in the way seen in this plant, if those two members originally lay side by side in the same spireme. The univalent chromosomes making up a bivalent, if attached at all during the open spireme, must have been attached end to end.

On the whole, therefore, the singular absence of parallelism at all stages, the clear relationship established between the second contraction loops and the bivalent chromosomes, and the regular linking of the latter during diakinesis, seem to make it quite certain that we are dealing here with chromosomes which are attached end to end in a univalent spireme.

The Position of the Chromosomes. The regularity with which the bivalent chromosomes are linked together in diakinesis raises an interesting question concerning the relative positions of the chromosomes in the nucleus. The fact that linking seems to conform to a definite scheme suggests that during the preceding spireme stage the individual chromosomes occupied the same relative position in all nuclei, being attached to each other at the same places and in the same way. How far back this condition existed is probably difficult, if not impossible, to determine in a plant where the individuality of the chromosomes is lost sight of during resting stages; but at least the interesting possibility is suggested that such a definite arrangement might be present in all nuclei. Whatever may be the situation in the somatic cells in general, it is quite probable that the chromosomes are arranged during the last archesporial mitosis according to a definite plan, so that they come to occupy the same relative positions in the subsequent prophase stages.

The Nucleolus. There is always at least one nucleolus in the nucleus of every pollen mother cell in *Oe. franciscana.* That its function seems to be at least partially that of storage is indicated by the appearance presented during late synizesis, when the threads surrounding and attached to the nucleolus become greatly swollen, as though gorged with material suddenly poured into them. At the same time, the nucleolus begins to lose its staining qualities and to look empty. The material which it stores stains intensely with the heamatoxylin, and would seem, therefore, to be a form of chromatin. The nucleolus itself, when divested of the chromatic contents, does not take the stain and would therefore seem to be composed of a substance perhaps closely related to, or identical with, linin. At different times it presents a somewhat different appearance. Before discharging its contents, it contains at times a crystalloid body. In other cases one or more vacuoles are present in the interior. After it has been emptied of its chromatin content, it very generally shows within a black-staining, spherical little body, the endonucleolus. This seems at times to be connected directly with the spireme by a prominent thread, but usually it lies freely in the nucleolus. It outlasts the nucleolus ordinarily, being occasionally seen at metaphase, but disappears in time without having undergone division, and seems therefore to have no part to play in the development of the nucleoli in the daughter nuclei. The fact that during interkinesis several new nucleoli can be seen to develop simultaneously in contact with the chromosomes, and probably from them, makes it seem probable that the nucleoli arise *de novo*, and not from preëxisting ones. The nucleolus seems, therefore, to be a body which stores material of some sort, probably chromatin, and which is formed anew in each nucleus.

Genetical

It has been very clearly pointed out by Davis (1915) that the chief source of doubt concerning the validity of many of the interpretations based on breeding experiments in the genus Oenothera lies in the ever-recurring suspicion that the species under consideration are not genetically pure. He has shown that the mere fact of breeding true is not a sufficient test of purity, in view of the large percentage of pollen and seed sterility often encountered in these plants, which phenomena introduce an element of doubt, in that they allow of the possibility of the presence of more than one type of gamete, only one of which may survive to function, or, if they all function, to produce successful progeny. The element of doubt thus introduced into the study of even those species which seem for the most part to breed true tends to cast a shadow of suspicion upon the conclusions based upon this work. It becomes, therefore, a matter of great importance to develop methods by which the purity of a species may be satisfactorily tested, and to find species whose purity is beyond question.

The main barrier in the way of determining whether the members of the *Lamarckiana* group are pure or impure is the high percentage of sterility there found. Until the reasons for this sterility are determined, it is impossible to tell whether "mutants" thrown by them are indeed mutants, or whether they are Mendelian segregates. Meanwhile, the abundant sterility is itself a possible indication of hybridity. There are, however, a few species of Oenothera in which the percentage of sterility is lower than in most of the *Lamarckiana* group, and which at the same time breed quite true. At least one of these species, however, *Oe. biennis* L., is not entirely above suspicion. While it breeds remarkably true in the main, it has nevertheless been shown by Stomps (1914) to throw a small percentage of "mutants." Furthermore, it shows over 50 percent pollen sterility. The other species, however, which may prove to be the long-looked-for pure species, are *Oe. grandiflora* and *Oe. franciscana*. Both of these are extremely stable, and at the same time show a very small percentage of both pollen and seed sterility.

The latter species has proved absolutely stable thus far, as is shown by table 1, published herewith through the kindness of Dr. Davis.

TABLE 1. *History of Oenothera franciscana B.*

Year	Culture	Source of Culture (Culture and Plant)	Description of Culture
1913.......	13.21	Bartlett 10_6–12_6	52 plants, uniform
1914.......	14.21	13.21*a*, *c*	18 plants, "
1915.......	15.21	14.21*a*	16 plants, "
1916.......	16.21	15.21	9 plants, "
1917.......	17.21	16.21*a*	12 plants, "
1918.......	——	——	No culture
1919.......	19.21	17.21, III–1	15 plants, uniform
1920.......	20.21	19.21, III–2	25 plants, "
1921.......	21.21	20.21, 5	1,352 mature plants 21 remaining as rosettes 1,373 uniform and typical
Total.....			1,520 uniform and typical

It will be seen from this table that during the eight years in which this strain has been carried along, not a single mutation or aberrant form has been discovered. There is very little pollen sterility, and the seed tests made by Dr. Davis (table 2) show that the percentage of seed sterility is also very small.

TABLE 2. *Seed Fertility in Oenothera franciscana B.*

Year	Culture	Procedure	Result of Test	Percentage of Germination
1915....	15.21	Contents of two capsules, seeds germinated in earth	442 seedlings 280 seed-like structures 722	61%
1919....	19.21	Contents of one capsule, soaked 24 hours. Subjected to alternate exhaust and pressure (50 lbs.) twice in 24 hours	398 seedlings 43 seed-like structures 441	90.2%
1921....	21.21	Contents of three capsules, soaked 24 hours. Subjected to alternate exhaust and pressure (30 lbs.) 7 times in 24 hours	1,425 seedlings 209 seed-like structures 1,634	87.3%

All the indications so far, then, point to *Oe. grandiflora* and *Oe. franciscana*, and especially to the latter species, as likely to prove genetically pure. In view of this fact, it becomes a matter of extreme interest to investigate these plants cytologically and to compare them from this standpoint also with the members of the *Lamarckiana* group. The maturation divisions in the pollen mother cells of *Oe. grandiflora* have been studied by Davis (1909). He made the interesting discovery that in this species the seeming incompatibility between homologous chromosomes, which is so characteristic of the plants of the *Lamarckiana* series, and which seems in their case to point strongly to a hybrid nature, is lacking. The homologous chromosomes pair with entire regularity, and are carried to the equatorial plate and separated in a perfectly typical manner. In view of this suggestive study, it becomes of interest also to make a cytological study of *Oe. franciscana*, which is even more strikingly uniform in its behavior than *Oe. grandiflora*. The results of this study have been very interesting as indicating an even more striking regularity of chromosome behavior during the maturation divisions than that found in *Oe. grandiflora*. Not only do the chromosomes regularly pair with one another, but they show a most interesting uniformity of position, resulting in the linking of bivalent chromosomes in a seemingly constant manner; and, most striking of all, a definite morphological peculiarity, in the nature of a closed circle of four univalent chromosomes, is found to be always present during diakinesis, a feature which is most readily observable, and whose constancy is a clear reflection of the uniformity which characterizes the activity of the chromosomes as a whole at this time.

It seems to be a fact, therefore, that the failure of homologous chromosomes to pair at diakinesis, and the tendency for the chromosomes to come to the equatorial plate in an irregular fashion, are not characteristic of the genus Oenothera as a whole, but only of those species which have been found to be unstable. In the stable species, homologous chromosomes seem to have a strong affinity for one another during diakinesis. In the unstable species, they seem to have little or no affinity for one another, and the most obvious inference is that this incompatibility is due to the hybrid nature of the species. Such an inference may not be correct, however, as it may turn out, when a careful cytological and physiological investigation is made of pollen and ovule development in the more unstable species, that there is but one type of gamete present for each sex. If such be the case, then the irregularity of chromosome behavior is to be considered rather a morphological expression of the peculiar condition, physiological or morphological, which results in the tendency to mutate.

However this may be, the correlation between hereditary instability and the irregular behavior of chromosomes on the one hand, and between stability and the regular, uniform behavior of chromosomes on the other, is rather striking. Not only does it emphasize the probable purity of the stable species, but by contrast it should make one all the more cautious about considering the unstable ones as genetically pure.

In conclusion, I wish to thank Dr. B. M. Davis for his great kindness in allowing me to make use of material growing in his gardens, in permitting me to include in this paper some of his unpublished data, as well as for the interest which he has manifested in the study as it has progressed.

Summary

1. The nucleus passes through a definite resting period of considerable length, previous to entering upon the heterotypic prophase. During this period all signs of the individual chromosomes are lost. The threads are unpaired.

2. The approach of prophase is indicated by occasional, though not very striking, parallelisms in the network, due to the contraction of threads here and there. There is no general pairing of threads such as has been described by many workers on other forms.

3. Prophase is ushered in by a general contraction of the reticulum, which soon causes the threads to be torn away from most of the periphery and to shrink into a tight knot—the synizetic knot.

4. During synizesis, many of the threads disappear and their contents pass into other threads, which thus become more and more prominent, and finally emerge from the knot as the spireme. There is very little parallelism of threads during synizesis, and what little there is seems to be due merely to the way in which the threads condense. There is no reason for believing that it represents the fusion of the separated halves of a univalent spireme, or the synapsis of two univalent spiremes to form a bivalent one.

5. There is a well marked "open-spireme" stage, during which the threads are fairly uniform in diameter and display no evidence of doubleness. There is no strepsinema condition, the nucleus passing into the second contraction by the gradual accumulation of material in the center of the nucleus, and by the contraction of the spireme thread into smaller and smaller loops radiating from the center, the threads composing these loops being single in nature.

6. The loops can be traced uninterruptedly through the second contraction, and undoubtedly form the bivalent chromosomes which emerge at the end of this period. The univalent chromosomes are arranged end to end, or telosynaptically, in these loops, the places where they join being marked by constrictions.

7. The bivalent chromosomes emerge in a definite way from the second contraction, and are arranged in such a manner that they form one closed circle, consisting of four univalent chromosomes, and five rings composed each of two chromosomes, all of which are linked together at first according to a definite scheme. Homologous chromosomes are perfectly compatible.

8. During diakinesis, these rings separate to a greater or less extent from one another. Each individual ring, however, including the circle of four chromosomes, remains intact.

9. The rings are drawn to the equatorial plate at metaphase. The circle of four chromosomes breaks into two pairs. The rings are arranged in a flat plate, and the univalent chromosomes are separated and carried to opposite poles without the least sign of irregularity.

10. The longitudinal split in the daughter chromosomes preparatory to the homoeotypic division does not appear until late interkinesis. During interkinesis, the individual chromosomes remain clearly visible, being attached in only the most meager way.

11. The homoeotypic division is in no respect unusual and is followed by the simultaneous cutting in of walls to form the four spores of the tetrad.

12. The nucleus of each spore passes into a resting condition in which all trace of the individual chromosomes is lost. This process is easily followed, and it is clear that no splitting of the chromosomes into two threads takes place, as has been described in the telophase of some plants.

13. The chromosomes in this plant are arranged telosynaptically, and form a univalent spireme during prophase of the heterotypic mitosis.

14. The reduction processes in *Oenothera franciscana*, which is genetically a very stable species, are extremely regular and typical, in striking contrast with what is found to be the case in the less stable species of the genus, but in agreement with the condition which has been found in one of the other stable species, *Oe. grandiflora*.

Goucher College

BIBLIOGRAPHY

Allen, C. E. 1905. Nuclear division in the pollen mother-cells of *Lilium canadense*. Annals Bot. **19**: 189–258.

Bartlett, H. H. 1914. Systematic studies on Oenothera IV. *Oe. franciscana* and *Oe. venusta*. Rhodora **16**: 33–37.

Davis, B. M. 1909. Cytological studies on Oenothera I. Pollen development of *Oenothera grandiflora*. Annals Bot. **23**: 551–571.

——. 1915. The test of a pure species of Oenothera. Proc. Amer. Philos. Soc. **54**: 226–245.

——. 1916. Hybrids of *Oenothera biennis* and *Oe. franciscana* in the first and second generations. Genetics **1**: 197–251.

Digby, L. 1910. The somatic, premeiotic, and meiotic nuclear divisions of *Galtonia candicans*. Annals Bot. **24**: 727–757.

——. 1912. The cytology of *Primula kewensis* and of other related Primula hybrids. Annals Bot. **26**: 357–388.

——. 1919. On the archesporial and meiotic mitoses in Osmunda. Annals Bot. **33**: 135–172.

Farmer, J. B., and Moore, J. E. S. 1903. New investigations in the reduction phenomena of animals and plants. Proc. Roy. Soc. London **72**: 104–108.

—— ——. 1905. On the meiotic phase (reduction divisions) in animals and plants. Quar. Jour. Micr. Sci. **48**: 489–557.

Grégoire, V. 1904. La réduction numérique des chromosomes et les cinèses de maturation. La Cellule **21**: 297–314.

——. 1907. La formation des gémini hétérotypiques dans les végétaux. La Cellule **24**: 369–420.

Sharp, L. W. 1913. Somatic chromosomes in Vicia. La Cellule **29**: 297–331.

——. 1920. Somatic chromosomes in Tradescantia. Amer. Jour. Bot. **7**: 341–354.

Stomps, T. J. 1914. Parallele Mutationen bei *Oenothera biennis* L. Ber. Deutsch. Bot. Ges. **32**: 179–188.

de Vries, H. 1915. The coefficient of mutation in *Oenothera biennis* L. Bot. Gaz. **59**: 169–196.

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida, using a Spencer high-powered binocular microscope, with Spencer 1.8 oil-immersion objective and 10X eye-piece. They were enlarged by the use of a pantograph, and reduced one fourth in reproduction. Magnification 2,280 diameters, except figure 35, which is at a magnification of 1,140.

Plate XXV

Figs. 1, 2. Resting stage before heterotypic prophase.

Fig. 3. Parallelism, due to contraction of threads here and there, indicating approaching prophase.

Fig. 4. Beginning of prophase.

Fig. 5. Network is beginning to contract to form synizetic knot.

Figs. 6, 7. Tangential sections during early synizesis, showing disappearance of some threads and augmentation of others.

Figs. 8, 9. Later synizesis. The threads becoming more uniform and thicker.

Fig. 10. Spireme well formed. Threads near the nucleolus gorged with material.

Figs. 11, 12. End of synizesis. Thread single and uniform except near the nucleolus, often beaded. Endonucleolus present.

Plate XXVI

Fig. 13. Same as last two figures. Nucleolus returning to the center.

Fig. 14. Open spireme stage. Notice connection between the endonucleolus and the spireme. (See also fig. 16.)

Fig. 15. Beginning of second contraction. Central portion of reticulum begins to thicken. Peripheral portion thrown into radiating loops.

Figs. 16–18. Later stages in condensation, the central region larger, the peripheral threads thicker and shorter. Note position of nucleolus in figure 18.

Fig. 19. Further stage in contraction. Note chromosome forming the distal end of a loop. The sides of two of the loops seem to have partially fused together. This stage is frequently seen.

Fig. 20. Loops, or bivalent chromosomes, radiating from the central region.

Fig. 21. The climax of contraction. Loops still plainly seen, however.

Fig. 22. Second contraction knot begins to loosen. Two rings at one end and one at the other.

Fig. 23. Slightly later stage. One loop at the top, two rings at the bottom of the figure. Usually at about this stage one or more bivalent chromosomes separate entirely from the rest. Note endonucleolus.

Fig. 24. Circle of four chromosomes, to which are linked two two-linked chains, each link being a pair of chromosomes.

Plate XXVII

Fig. 25. Circle of four, with one two-linked chain attached. Three rings separated.

Fig. 26. Circle of four, with three rings attached.

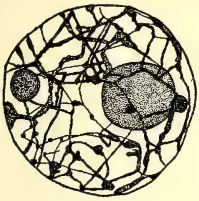
Fig. 27. Circle of four, with one two-linked chain, and one single ring attached.

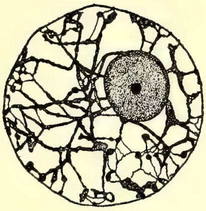
Fig. 28. Circle of four, and five separate rings. Mid-diakinesis. Nucleolus melting.

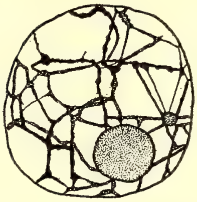
Fig. 29. Multipolar spindle. The circle of four unbroken. Two rings still linked to it. Endonucleolus still present.

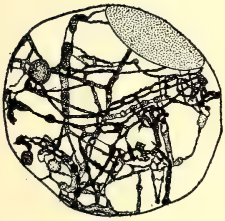
Fig. 30. Metaphase of the heterotypic mitosis. Note regularity of arrangement.

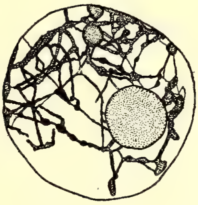
Fig. 31. Anaphase of the heterotypic mitosis.

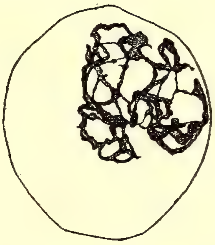

1

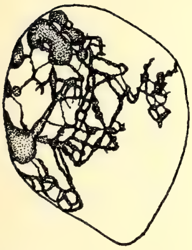

2

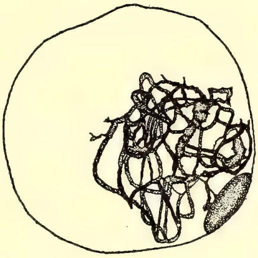

3

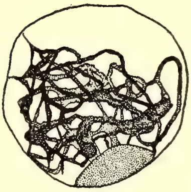

4

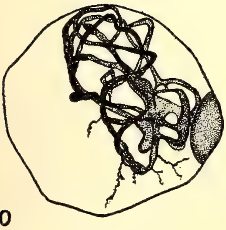

5

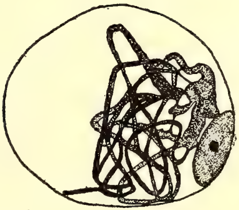

6

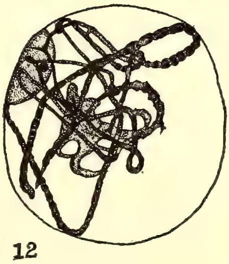

7


8

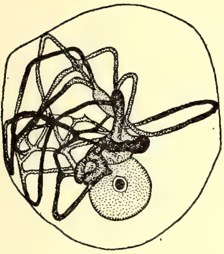

9


10

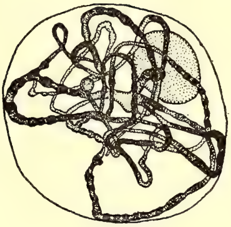

11


12

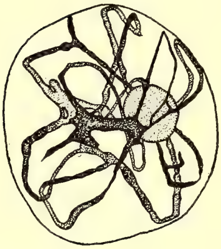
CLELAND: OENOTHERA FRANCISCANA



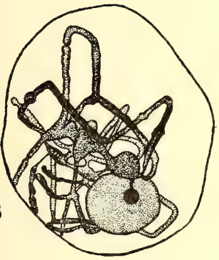
13



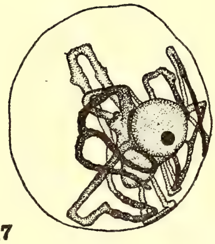
14



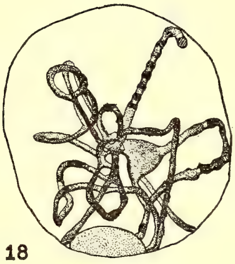
15



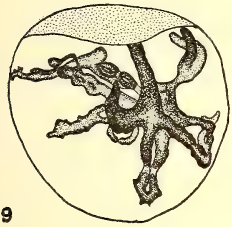
16



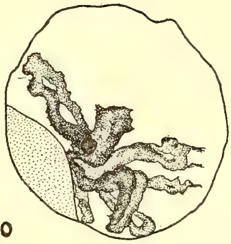
17



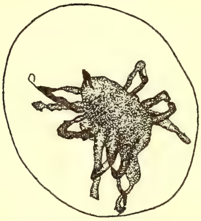
18



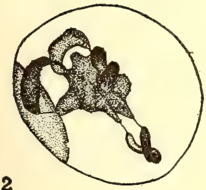
19



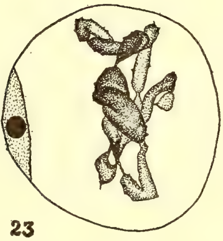
20



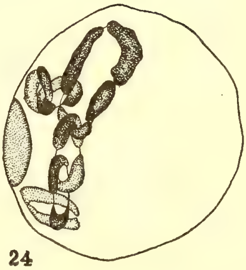
21



22

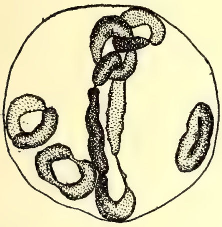


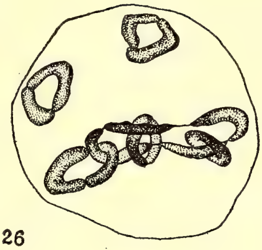
23

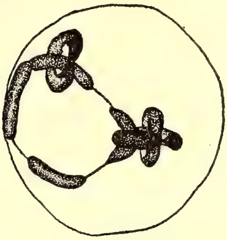


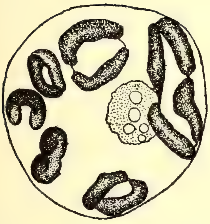
24

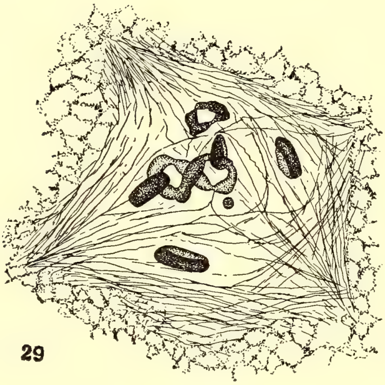
Cleland: Oenothera franciscana

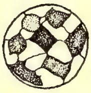

25

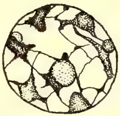

26

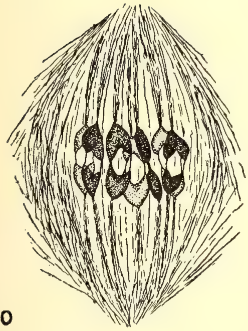

27

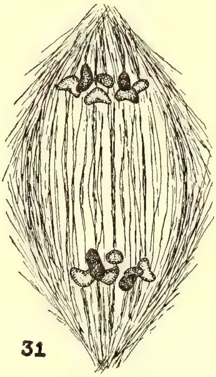

28

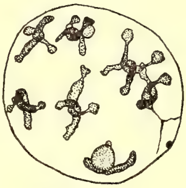

29

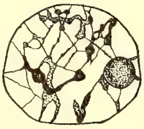

32

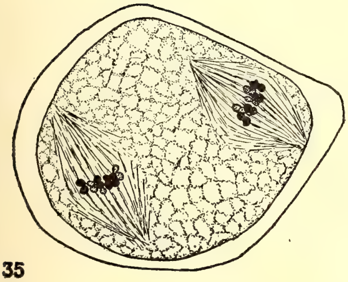

33

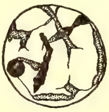

30

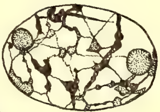

31


34


37


35


36


38

CLELAND: OENOTHERA FRANCISCANA

FIG. 32. Early interkinesis.

FIG. 33. Later. Chromosomes distinct, but to all appearances as yet unsplit.

FIG. 34. Late interkinesis. Chromosomes have split, and halves have bent around each other.

FIG. 35. Metaphase of the homoeotypic mitosis. × 1,140.

FIG. 36. Granddaughter nucleus, soon after formation. Chromosomes are beginning to lengthen and to send out delicate projections, which later fuse to form a network.

FIG. 37. Chromosomes are rapidly losing their identity, as their contents pass out into the projecting threads.

FIG. 38. Resting stage in a granddaughter nucleus. Chromosomes are entirely obscured.

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1922

President: HENRY C. COWLES
University of Chicago

Vice-President: MARGARET C. FERGUSON
Wellesley College

Secretary: I. F. LEWIS
University of Virginia, University, Va.

Treasurer: IRVING W. BAILEY
Bussey Institution, Harvard University

Councilors:

N. L. BRITTON
New York Botanical Garden

J. C. ARTHUR
Purdue University

C. E. ALLEN
University of Wisconsin

Any one actively interested in botanical work is eligible for membership. Candidates for membership must be recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including one subscription to the AMERICAN JOURNAL OF BOTANY. Candidates proposed for membership, and approved by the Council, may receive one copy of the JOURNAL at $4.00 per year pending their election at the winter meeting of the Society.

Had You Ever Thought This About The Greenhouses We Build

Had you ever thought that if we didn't use quite such ornamental hoods over the doors; and made the eaves straight instead of curved, that although the house might not be quite as attractive it would be just as good a growing house and exactly as durable?

In other words, the kind of upholstery in a car has no effect on the hill-climbing power of the engine.

To all of which we heartily agree.

Furthermore, we build just such a house as you describe.

It's called our Practical Purpose house.

Glad to give you figures just to show you how decidedly practical it is when it comes to the price.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

PHILADELPHIA
133 So. 12th St.

Vol. IX. OCTOBER, 1922 No. 8

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

CONTENTS

Studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in seed plants. . . . . THOMAS WYATT TURNER 415

A preliminary notice of genetical studies of resistance to mildew in Oenothera. PETER J. KLAPHAAK and H. H. BARTLETT 446

Growth of some parasitic fungi in synthetic culture media. H. C. YOUNG and C. W. BENNETT 459

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT PRINCE AND LEMON STS., LANCASTER, PA.
LONDON AGENTS
WHELDON AND WESLEY, LTD.
28 ESSEX STREET, STRAND, LONDON, W. C. 2

Smithsonian Institution
OCT 20 1922
National Museum

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
The Thompson Institute For Plant Research, Inc.

H. H. BARTLETT,
University of Michigan

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ARTHUR H. GRAVES,
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

I. F. LEWIS,
University of Virginia

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Postage to Canada, 20 cents; to other foreign countries (except Mexico and Cuba) 40 cents a volume on annual subscriptions. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume, post free.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or The New Era Printing Co., Inc., Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. IX OCTOBER, 1922 No. 8

STUDIES OF THE MECHANISM OF THE PHYSIOLOGICAL EFFECTS OF CERTAIN MINERAL SALTS IN ALTERING THE RATIO OF TOP GROWTH TO ROOT GROWTH IN SEED PLANTS

THOMAS WYATT TURNER

(Received for publication December 14, 1921)

INTRODUCTION

Very few researches are recorded in the field of plant physiology which deal specifically with top-root growth ratios in plants and with the factors that may alter such ratios. In attacking this problem, great difficulties are unavoidably encountered because of the complexity of the medium in which experiments with roots must always be carried on. From the standpoint of physical chemistry, our knowledge as to reactions between plant roots and the mineral nutrient solutions in which they may be growing is not much more perfect than that of reactions among plant roots, soil particles, and soil solution.

The scientific plant grower as well as the physiologist would be greatly aided in their respective fields if there could be furnished a more exact knowledge of the interrelations between the aërial and the subterranean plant parts: as to how intimately interdependent these members are, and also to what extent and under what conditions either may behave independently of the other.

Getting results of physiological value in respect to this problem from investigations with woody plants is a slow and tedious undertaking, yet the field of horticulture and pomology through its numerous experiments in pruning and grafting contains much valuable material which clearly indicates the varying relationships of tops and roots under varying conditions of nutrition and treatment, as well as the necessity of more exact physiological investigations seeking to get at the causes. The fertilizer problem will not be solved either from a scientific or from a practical point of view until more definite information is obtained as to how the particular fertilizer used affects the plant roots, or as to how to localize the effect of a particular treatment in the desired organ.

The investigations discussed in this paper are concerned chiefly with the effects of nitrate ions upon the relative behavior of tops and roots in

[The **Journal** for July (9: 339–413) was issued August 30, 1922].

certain plants. There has long been a well defined impression that nitrates check the growth of roots; whether this supposed checking is the result of the action of the salt ions directly upon the roots, or whether the effect is brought about indirectly because of a much more stimulating effect of the salt ions upon the aërial portions of the plant, has been left, in a large measure, unanswered.

STATUS OF THE PROBLEM AS PRESENTED IN THE LITERATURE

Moeller (19) was one of the first workers to call attention to the difference in ratio between tops and roots of plants as they grew in solutions of different concentrations. The more dilute solutions which he used were such that they brought about, as he states, dwarfing of the plants growing in them. It would seem, therefore, that his results should be attributed more to starvation effects than to any definite consequence of the concentration as such. He found that weight of tops was five times that of roots in the normal plants, while in the dwarf cultures the top weight was only twice that of roots. The root systems of the dwarf plants, however, were absolutely smaller than those of the normal.

In 1906, Livingston (17) worked upon the relation between roots and tops in wheat. He grew his cultures in soil, using a "poor" soil from Takoma Park, Md., as the basis. In some of his experiments he used the "poor" soil untreated, in others he added stable manure ranging from 5,000 p.p.m. to 50,000 p.p.m.[1] He drew the conclusion from his work that variation in tops is correlated with the number and length of lateral roots. He found that poor soil inhibits growth of lateral roots and also brings on a premature aging of the cortical cells, both of which conditions inhibit the intake of water. He thus explains the dwarfing of tops in his "poor"-soil plants largely on the basis of an inadequacy of water. Soil cultures are not wholly comparable to water cultures, yet the poverty of certain mineral nutrients in water cultures will bring about a lowered ratio of tops to roots, as Livingston found in his "poor"-soil experiments, and in such case there is no evidence that a deficiency of water supply is a factor. In a soil devoid of nutrients, there would be, undoubtedly, retardation of the growth of both tops and roots.

Harris (12), working in 1913 at Cornell University, sought to determine to what extent the ratio of tops to roots in certain plants is affected by moisture, plant food, and age. He grew plants in aqueous extracts of soil and in sand in which moisture and fertilizer were controlled. His results indicate that all three factors considered in the experiment influence the ratio of tops to roots in a positive way. His experiments with plants grown in solution and in soil, while in no way conclusive as to the relative parts played by moisture and fertilizer in altering ratios of tops to roots, are very suggestive. A brief table (table 1) of some of his results will bring out very well the conclusions.

---

[1] P.p.m. = parts per million.

TABLE 1

Fertilizer Treatment	Moisture in Soil	Ratio of Tops to Roots at Maturity (Wheat)
None	30%	8.1 : 1
None	15%	3.1 : 1
Complete	30%	8.7 : 1
Complete	15%	4.1 : 1
Complete with high nitrogen	30%	9.8 : 1
Complete with high nitrogen	15%	2.9 : 1

It will be seen from table 1 that fertilizer and moisture appear to increase the ratio of tops to roots, but that the fertilizer factor plays apparently a small rôle in comparison with moisture.

More or less recently three workers, Tottingham (31), Brenchley (2), and Stiles (29), employing nutrient solutions, have given considerable attention to the relative behavior of tops and roots of plants growing in solutions. But such attention as was given was only a side issue and not the primary aim of their investigations. Because of certain points of similarity running through the works of these three authors, it seems advisable to discuss them together. Both Stiles and Miss Brenchley sought to determine the effect upon growth of certain plants by varying the concentration of the nutrient solution. They disagree sharply as to the range of concentration of nutrients in a solution which will offer favorable growth for plants, yet their results agree fairly well in respect to what they interpret as the effect of concentration on the ratio of tops to roots, and the fact has been pointed out more than once in their works that as the solution decreases in concentration, the ratio of dry weight of tops to roots also decreases. Table 2, taken from their results, brings out these findings.

TABLE 2

Concentration	Full Strength	1/5	1/10	1/20	Duration	Treatment
Stiles						
Series 1, $\frac{\text{Tops}}{\text{Roots}}$	4.0	3.7	3.4	3.0	39 days	Changed often
" 2, $\frac{\text{Tops}}{\text{Roots}}$	3.0	3.1	3.3	2.5	41 days	Changed twice
" 3, $\frac{\text{Tops}}{\text{Roots}}$	2.7	3.2	3.1	2.2	42 days	Changed once
Brenchley						
Series 1, $\frac{\text{Tops}}{\text{Roots}}$	2.7	2.5	2.4	2.5	49 days	Changed often
	3.7	2.7	1.8	1.5		Changed once
	2.6	2.2	1.8	1.2		Changed never
" 2, $\frac{\text{Tops}}{\text{Roots}}$	3.3	3.0	2.3	1.9	49 days	Changed often
	2.8	2.3	1.8	1.4		Changed once
	2.6	1.8	1.3	1.0		Changed never
" 3, $\frac{\text{Tops}}{\text{Roots}}$	3.9	3.1	2.8	2.4	49 days	Changed often
	2.3	2.3	1.9	1.6		Changed once
	1.9	1.3	1.2	1.0		Changed never

The full-strength solution employed by Stiles had a total salt concentration of 1,830 p.p.m., while that used by Miss Brenchley contained 3,040 p.p.m. In their investigations both workers grew plants in the full-strength solution and in three dilutions of the same (1/5, 1/10, and 1/20 of the full strength).

In making a critical examination of the results given in table 2 in connection with the dilutions employed, as well as in the light of the possible behavior of salts in very dilute solutions, the question arises as to whether these results are really significant in relation to a varying concentration as such, or whether or not they may be indications of a deficiency of one or more ions. In order that experiments may be conclusive on this point, ample precautions should be taken that the minimum requirements of essential salts are at hand throughout the duration of the experiment, otherwise we may have the lack of some salt as the limiting factor. Certain results to be reported later in this paper show that increasing the concentration alone in a solution, there being adequate nutrients present otherwise, will not give higher ratios of tops to roots than result in another solution with even lower concentration of total salts but of different chemical composition.

As further evidence that concentration in itself is not a controlling factor, table 3, computed from table 15, page 187, of Tottingham's work (31), will be of interest. While the original paper in question does not give the ratios, it does give dry weights of tops and of roots from which the ratios have been calculated. It should be added that these results of Tottingham's are based mostly upon single cultures, and this fact should be considered in evaluating the data.

TABLE 3

	Total Concentration of Salts in Solution (%)							
	.05	.10	.15	.20	.25	.30	.35	.40
Ratio, $\frac{\text{Tops}}{\text{Roots}}$	3.22	3.13	3.17	2.58	3.17	3.03	3.5	3.11

	Total Concentration of Salts in Solution (%)							
	.45	.50	.55	.60	.65	.70	.75	.80
$\frac{\text{Tops}}{\text{Roots}}$	2.91	2.84	3.10	3.00	3.00	3.03	2.91	3.10

In the experiment upon which the table is based, the total concentration of the solution (a modified Knop's solution) varies, as will be noticed, from 500 p.p.m. to 8,000 p.p.m., with no influence whatever, as the figures show, upon the ratios of top to root growth of the wheat plants employed. In another phase of his researches, Tottingham held the concentration constant, but varied the salt proportions of his solution chiefly around monobasic potassium phosphate, which constituted from one tenth to seven tenths of the total salt concentration employed. He actually made up his solutions on the basis of osmotic pressure (.6 percent solution corresponding to an osmotic pressure of 2.50 atmospheres), but we may, for convenience, use total concentrations in the discussion, since for these dilute solutions osmotic pressure varies with the concentration.

Tottingham's variations in the proportion of salts gave a total of 84 solutions. In 28 of these, one tenth of the total concentration was due to monobasic potassium phosphate; in 21 solutions, two tenths; in 15, three tenths; in 10, four tenths; in 6, five tenths; in 3, six tenths; in 1, seven tenths. Cultures were grown in each of the solutions. Using the figures on his triangular diagrams, the top-root ratios have been averaged for at least ten cultures from each of those percentages of monobasic potassium phosphate, giving ten or more solutions, while ratios for all the cultures have been averaged for the remaining triangles. The results are again very suggestive (table 3*a*).

TABLE 3*a*

	Partial Concentration of KH_2PO_4 in a .6% Solution						
	.1	.2	.3	.4	.5	.6	.7
$\frac{\text{Tops}}{\text{Roots}}$	3.1	2.84	2.69	2.57	2.77	2.43	2.42

With the exception of one result, the ratios of tops to roots shown in table 3*a* vary inversely with the concentration of monobasic potassium phosphate in the solution. Whatever importance we may attach to this table, it stands out in contrast to table 3 in emphasizing the importance of the composition of the nutrient solution in affecting top-root ratios.

Cameron (5) and others have called attention to the fact that the relative proportion of mineral constituents has a marked effect on growth, causing tops to grow relatively faster than roots in some cases, at other times causing roots to make the more rapid growth.

The step from a plant like wheat or barley grown in a nutrient solution, to a deciduous orchard plant growing in the soil, is not an immediate one, but it might not be out of place to mention here some pruning results of Chandler (6) in so far as they concern the ratio of top to root growth in certain orchard trees. He gives data supporting the following statements:

> While the nitrate has increased both top and root growth, it has not increased root growth to as large an extent as it has increased top growth. Thus, while the top growth of fertilized trees [peaches] is twice as great as that of the unfertilized trees, the root growth is only 50 percent greater.

Statement of the Problem

It is clear from the foregoing brief discussion and review of literature that the few investigations reported up to the present time which deal with top-root growth ratios in plants as they are influenced by nutrient concentrations, give inconclusive results. It has not been possible to say with any degree of exactness just which one of several factors has been predominant in a given experiment. It has been found that water, temperature, light, and shade will influence top-root ratios; but this paper is confined to the effect of nutrient solutions. Miss Brenchley (3) has recently obtained some suggestive results by controlling the temperature factor in connection with nutrient cultures, and she shows how very important this factor is, especially with reference to the roots.

In view, therefore, of the scarcity of significant data, the writer has undertaken to investigate the influence of certain mineral nutrients in altering the ratio of top to root growth in certain plants and to suggest the probable mechanism underlying the resulting responses. This is a new field for specific investigation, and the solution of the problems involved offers much of value to the science of plant physiology, as well as to agricultural practice.

To a considerable extent, roots have specific nature and habits of their own apart from any characteristics which they may have as co-workers with tops for the good of the whole plant; they may be sharply polymorphic, as discussed later in this paper, and it has been suggested that they may behave at times as saprophytes when supplied with suitable carbohydrates, living at the expense of tops.

Materials and Methods

Since these experiments were concerned with the effect of nitrates, a prerequisite at the outset was to devise a solution which would allow a wide variation in the nitrate ions without affecting very much the other essential elements necessary for favorable plant growth. One has ordinarily a wide choice in the selection of a nutrient solution; the general point of view, as gathered from a study of the solutions used by the various investigators and from their statements, is that there is no "best solution" nor specific ratio of ions or elements which will insure an optimum yield of plants under all conditions. Solutions, as mentioned already, which give optimum yield of roots, may not and often do not give optimum yield of tops, if by "optimum" we mean greatest green weight or dry weight.

It has been shown that, provided the total supply of mineral nutrients is adequate, the toleration of plants may extend over wide limits without significant falling off in yield.

The problem of varying nitrate ions for the present study without affecting greatly the other components was met by combining calcium nitrate, potassium nitrate, and magnesium nitrate as the source of nitrates; monobasic potassium phosphate, magnesium phosphate, and monobasic calcium phosphate as the source of phosphates; and magnesium sulphate, potassium sulphate, and calcium sulphate as the source of sulphates. The basic elements except iron are likewise derived from three different compounds. This really amounts to a three-salt solution. The following scheme will better enable us to visualize the component salts in the solution:

$Ca(NO_3)_2$	KNO_3	$Mg(NO_3)_4$
$Ca(H_2PO_4)_2$	KH_2PO_4	$Mg_3(PO_4)_2$
$CaSO_4$	K_2SO_4	$MgSO_4$

Reduction of salts in a horizontal or a vertical direction will enable us to vary the anions or cations at will. If a reduced or increased amount of nitrates is required in a particular solution, it may be secured by taking reduced or increased proportionate amounts of the three nitrate salts; while this will slightly reduce or increase, as the case may be, the total quantities of magnesium, calcium, and potassium in the solutions, the reductions or increments, being only a small part of the total amounts, would not greatly affect the total balance of these cations in the solution.[2] Care was observed always as to the total concentration.

In the first experiment, three solutions were employed; in these the quantities of nitrates were varied while the other components were kept approximately constant. This, of course, made a difference in the total concentration of the solutions, but the variations are well within limits which have been shown experimentally to be without significant effect upon yield.

The solution with the lowest nitrate concentration was designated Solution I; that next in order of concentration of nitrates, Solution II; and the one with the highest concentration of nitrates, Solution III. They had the following salt compositions:

	Sol. I (Low N)	Sol. II (Medium N)	Sol. III (High N)
KNO_3	.025 g.	.25 g.	.50 g.
KH_2PO_4	.1	.1	.1
K_2SO_4	.1	.1	.1
$Ca(NO_3)_2$	.025	.25	.50
$Ca(H_2PO_4)$	.1	.1	.1
$CaSO_4$	.1	.1	.1
$Mg(NO_3)_2$	.025	.25	.50
$Mg_3(PO_4)_2$	.1	.1	.1
$MgSO_4$	.1	.1	.1
H_2O	1,000 cc.	1,000 cc.	1,000 cc.
Total salts per liter	.675 g.	1.35 g.	2.1 g.

---

[2] This method of varying the salt components was suggested by Dr. Otis F. Curtis and was found to be a much more practical way of varying the amounts of, or omitting, an element than the usual practice of substitution in which entirely new elements are often introduced or the concentration of certain of the other ions already present is greatly altered.

The concentrations employed, as seen above, were 675 p.p.m., 1,350 p.p.m., and 2,100 p.p.m. As ordinarily used, Pfeffer's solution contains 1,500 p.p.m.; Knop's, 1,400 p.p.m.; Crone's, 2,000 p.p.m.; and Sachs', 2,700 p.p.m. Solutions II and III as used in these experiments compare favorably in total salt concentration with those of Knop and Crone, respectively.

Baker's analyzed C.P. chemicals were employed. Each salt was dissolved separately in a proportionate part of distilled water, and these components were mixed as needed; when a salt was difficultly soluble it was added in the form of a suspension. Ferrous sulphate was used as the source of iron, and was added directly to cultures when set up, and at each change of solution, at the rate of .002 gram per liter.

The hydrogen-ion concentration of each solution was carefully checked by the colorimetric method each time solutions were prepared. In making up the standard solutions, Merck's specially prepared sodium phosphate and potassium phosphate, purified as specified by Sörensen, were used. The results were not compared finally with electrometric determinations, but by using overlapping indicators as suggested both by Prideau, and by Clarke and Lubbs, approximate accuracy was assumed.

There was some variation in the hydrogen-ion concentration from time to time; this was attributed to the varying states of purity in the monobasic calcium and potassium phosphates, different lots of which had to be used as the work progressed. However, the pH value at the beginning of the first experiment was 6.47; and the range was never greater than from 5.59 to 6.47.

The plants grew well in these solutions and produced, as far as could be judged from appearances, healthy tops and roots over a period of 56 days, the longest duration of any set of experiments.

Cultures were grown in the three solutions simultaneously. Mason fruit jars which have a capacity of about 1,000 cc. were employed as containers. The screw-top type of jar was found to be the most practical kind for this work. It has the advantage that discs of paraffined manila paper may be inserted in place of the glass cap, which can then be made fast by screwing the metal band down tightly. The discs, held in this way, make a firm and stable support for the growing seedlings, and the operation entails very much less labor than the usual method of fastening the paper with rubber bands, or setting the seedlings in slit cork stoppers. Wire supports were made fast around the neck of the jar for support of the seedlings as they grew. Four cultures of each solution with four plants to a culture constituted a series.

The seeds were germinated between moist blotting paper; when the radicles of seedlings to be used were about one inch in length, four holes were made through the paraffined discs with a clean, pointed glass rod, through which they were inserted into the solution.

The solutions were changed once a week, but distilled water was added every other day to make up for water which may have passed off through transpiration or evaporation. At the beginning of the experiments, it was thought advisable to change the solutions more frequently than once a week because of a possible exhaustion of nitrates, particularly in the low-nitrate solution; however, qualitative tests for nitrates by the diphenylamine method made at intervals of two days showed that this precaution was not necessary. All experiments were carried on in the greenhouse of the department of plant physiology of Cornell University.

After a series of preliminary experiments with different plants (including peas, corn, barley, buckwheat, and flax), barley was selected for the first work as it showed in comparison with the others the slightest apparent variation under greenhouse conditions; both the cultures as a whole and the individuals in a culture made a fairly uniform development.

The variety of barley used was known as Silver King; it was secured from Professor H. H. Love of the department of plant breeding and was a pure line which had been selfed for several generations.

It was assumed that any marked variations in growth would be due to the varying amounts of nitrate at the disposal of the plant, since the other components were approximately equal in the three solutions and since the plants grew in the greenhouse under the same external conditions of light, temperature, and relative humidity.

The general method employed for obtaining quantitative data was to harvest a series from each solution at the same stated time, to wash off any salt crystals that may have adhered, then to separate carefully tops from roots and desiccate them at a temperature of 102° C. to constant weight. Other features such as length and branching were sometimes taken into consideration. In later experiments green weights were also considered.

EXPERIMENTAL DATA

Experiment I

The duration of this experiment was 49 days. It was begun August 23, 1920. The first series was harvested at the expiration of 14 days, and another was taken down every seven days thereafter until all were removed.

Table 4 gives the comparative results of the six series conducted with barley. Since growth has been quite uniform, the culture has been taken as the unit for calculation. The ratios of tops to roots per culture have been computed, as well as the probable errors of the means of these ratios for each series. In some cases the difference between the means of the ratios has been given together with the probable error of these differences. This will serve to show the significance of results obtained by adding nitrates. As an aid in comparing the absolute efficiencies of the three solutions, total dry weights of the four cultures are given also.

In comparing the ratios of tops to roots for the 14-day period (table 4), it is to be noted that there is no significant difference between the mean of cultures of the low-nitrate and that of the medium-nitrate solutions. The mean of the solution with high nitrate, however, differs by a margin that is quite significant. The ratio is shown here to increase with the nitrate concentration of the solution, and it appears also that the effect may be noticeable during the early period of growth, even before the seedling has exhausted the supply of food stored within the endosperm.

When we compare the actual dry weights in this series, the growth values of tops in the higher-nitrate solution are found to be greatest; in fact, the top weights show a variation in proportion to the nitrate concentration of the solutions. This is not true of roots. Using dry weights of the lowest nitrate solution as a basis, the top weight in the high-nitrate solution shows an increase of 28 percent over that in the low-nitrate solution, while the root weight shows an increase of only 2 percent; in the medium-nitrate solution, tops and roots show increases of about 15 percent each.

The relative behavior of tops and roots of those plants harvested at the expiration of 21 days is still more striking than in the case of those harvested after 14 days. The increase in dry weight of tops in the high-nitrate solution over that in the low is 23 percent, while the corresponding increase for roots is only .3 percent. Comparing these figures with 28 percent and 2 percent respectively in the preceding series, it becomes clear that roots are not being influenced in any such positive way by the nitrate treatment as tops. The medium-nitrate solution gives an increase of 13 percent and 10 percent respectively for tops and roots over the corresponding parts in the low-nitrate solution. This solution is regarded as the best balanced of the three and should show, therefore, more normal relationships between tops and roots.

Series 3 was harvested after 28 days' growth; significant differences are clearly shown between the ratios expressing the variation between different stages of maturity, as well as between the ratios of tops to roots in the different solutions. Notwithstanding the differences in mean ratio, there is not a striking difference between the total weights of cultures (tops and roots) of the different solutions, as a study of the table will show. This would indicate, of course, that the growth-producing values of the solutions do not differ very much.

Another interesting fact shown in Series 3 is that while ratios of tops to roots have increased steadily with the nitrate concentration of the solution, the actual dry weights of roots in the medium- and high-nitrate solutions have now become less in value than those of roots in the low-nitrate solution, and this relationship continues during the rest of the experiment.

The results of Series 4, 5, and 6 are given in table 4. They were harvested after 35, 42, and 49 days respectively, and bring out substantially the same facts which have been already emphasized, namely, that at all stages there is an increase in ratio of tops to roots as the nitrate content is increased.

In addition to the unmistakable evidence given as to the effect of increasing the nitrate concentration in the solution, as a study of the probable errors of the differences will show, attention might be called again, in Series 6, to the absolute dry weight of roots in the low-nitrate solution (1.8131 grams) and in the high-nitrate solution (1.1687 grams). The production of tops has been greatest in the medium-nitrate solution, as has been true throughout. If we now add total dry weights again for each of the three solutions, the interesting fact stands out that there is not a great difference in the total growth-producing value. The medium-nitrate solution is most efficient, with a total production of 13.2377 grams, followed by the high-nitrate solution with 11.7172 grams, and the low-nitrate solution with 11.4555 grams. The two latter solutions have about the same total efficiency, yet the ratio of tops to roots is strikingly different, the difference of the means being 3.75 ± .238, or about 70 percent.

Increase in Ratio of Tops to Roots as Plants Grow Older

The ratio of tops to roots increased both with nitrate concentration of the solution and with increased age. The normal increase of this ratio with age has not been adequately considered heretofore in the literature, and as yet the exact mechanism underlying this phenomenon awaits rational explanation. Table 4*a* gives data showing the increase with age; while the increases are quite significant in the low-nitrate solution, they are strikingly so in the medium- and high-nitrate solutions.

Growth Features

Quantitative data were not recorded as to transpiration or length of tops in Experiment I, but notes kept throughout the time the plants were growing show very little difference either in greenness or in height of tops. Plants grown in the low-nitrate solution were somewhat more slender, but appeared always more turgid. The aging or dying of the first or oldest leaf is not without interest. In the present experiment, as well as in the preliminary ones with barley, gradual dying of the lower leaf beginning with the tip started first with plants in the high-nitrate solution and would not begin in the low-nitrate solution until two or three weeks later.

The longest roots occurred in the high-nitrate solution. These were less branched, and the root system appeared, therefore, more slender than those in the other two solutions. It was particularly noticeable that aërial roots were scarcely formed at all in the high-nitrate cultures, while they developed abundantly in the others. The less abundant production of lateral roots in the high-nitrate solution as found here is in contrast to Müller-Thurgau's finding that the presence of nitrogen in a solution causes a much more vigorous growth of secondary roots.

As cultures grew in the greenhouse, they would be attacked during the later stages by *Erysiphe graminis;* the high-nitrate cultures were particularly susceptible to this fungus and could be readily picked out from the others by this fact. Medium-nitrate cultures were not so badly affected, and the low-nitrate cultures scarcely at all. This susceptibility to the fungus, varying with the nitrate concentration in the nutrient solution, was constant for the three sets of experiments conducted with barley and was independent of the total concentration. There seems to be a direct relation between the nitrate concentration and the plant's susceptibility to the fungus in question. Experiments are being conducted to throw some light on the mechanism of this relationship.

TABLE 4. *Experiment I (Barley); Ratio of Tops to Roots as Nitrate Concentration is Increased (Dry Weights in Grams)*

	Sol. I (Low Nitrate)			Sol. II (Medium Nitrate)			Sol. III (High Nitrate)		
	Tops	Roots	Tops / Roots	Tops	Roots	Tops / Roots	Tops	Roots	Tops / Roots
Series 1, *14 days*	.2145	.0644	3.33	.2180	.0690	3.159	.2462	.0635	3.88
	.1606	.0510	3.15	.2000	.0627	3.189	.2320	.0512	4.53
	.1830	.0517	3.54	.1960	.0524	3.740	.2179	.0583	3.74
	.1620	.0525	3.09	.2135	.0670	3.186	.2234	.0510	4.38
Total ..	.7201	.2196		.8275	.2511		.9195	.2240	
Mean ..			3.28 ± .068			3.32 ± .095			4.13 ± .129
Difference.....................High Nitrate − Medium Nitrate = .81 ± .160.									
Series 2, *21 days*	.3079	.0722	4.26	.4266	.1008	4.23	.4500	.0813	5.53
	.3525	.0845	4.17	.4287	.1050	4.08	.5591	.1105	5.06
	.4691	.1074	4.37	.4487	.1030	4.36	.5000	.0979	5.11
	.3882	.0953	4.07	.4045	.0861	4.70	.3625	.0709	5.11
Total ..	1.5177	.3594		1.7085	.3949		1.8716	.3606	
Mean ..			4.22 ± .0431			4.34 ± .088			5.20 ± .074
Difference.....................High Nitrate − Medium Nitrate = .86 ± .115.									
Series 3, *28 days*	.8500	.1889	4.50	.9100	.1675	5.43	.9500	.1700	5.59
	.7645	.1700	4.49	.8155	.1573	5.12	.9200	.1477	6.23
	.6814	.1552	4.39	.8965	.1770	5.07	.8880	.1500	5.92
	.8697	.2121	4.10	.9175	.1878	4.89	.8251	.1559	5.29
Total ..	3.1656	.7262		3.5395	.6896		3.5831	.6236	
Mean ..			4.37 ± .063			5.13 ± .076			5.75 ± .136
Difference.......................High Nitrate − Low Nitrate = 1.38 ± .149.									

TABLE 4 (*Continued*)

Series 4, *35 days*	1.2889	.2600	4.95	1.3420	.2026	6.62	1.4853	.2200	6.75
	1.2836	.2864	4.48	1.5517	.2233	6.94	1.4250	.2145	6.64
	1.2500	.2386	5.24	1.5643	.2381	6.56	1.1300	.1568	7.21
	1.1891	.2333	5.09	1.2883	.2099	6.13	1.3756	.2000	6.87
Total . .	5.0116	1.0183		5.7463	.8739		5.4159	.7913	
Mean . .			4.94±.111			6.56±.102			6.87±.083

Difference . . . . High Nitrate − Low Nitrate = 1.93 ± .138.

	Sol. I (Low Nitrate)			Sol. II (Medium Nitrate)			Sol. III (High Nitrate)		
	Tops	Roots	$\frac{\text{Tops}}{\text{Roots}}$	Tops	Roots	$\frac{\text{Tops}}{\text{Roots}}$	Tops	Roots	$\frac{\text{Tops}}{\text{Roots}}$
Series 5, *42 days*	1.9081	.4140	4.61	2.4047	.3131	7.70	2.0086	.2588	7.76
	1.6279	.3344	4.87	1.9157	.2387	8.02	1.7675	.2263	7.81
	1.6866	.3025	5.58	2.0000	.2700	7.41	2.1588	.3072	7.03
	1.8381	.3735	4.90	2.0960	.2717	7.71	1.9140	.2384	8.03
Total . .	7.0607	1.4244		8.4161	1.0935		7.8489	1.0307	
Mean . .			4.99±.139			7.71±.084			7.66±.146

Difference . . . . High Nitrate − Low Nitrate = 2.67 ± .201.

Series 6, *49 days*	2.5525	.4710	5.42	2.8500	.3243	8.79	2.7418	.3200	8.56
	2.3430	.4397	5.33	3.0170	.3619	8.33	3.2016	.3600	8.89
	2.3285	.4180	5.57	3.2385	.3952	8.19	2.1793	.2168	10.05
	2.4184	.4844	4.99	2.7052	.3456	7.82	2.4268	.2719	8.81
Total . .	9.6424	1.8131		11.8107	1.4270		10.5495	1.1687	
Mean . .			5.33±.082			8.28±.135			9.08±.224

Difference . . . . High Nitrate − Low Nitrate = 3.75 ± .238.

TABLE 4*a*. *Increase in Ratio of Tops to Roots in Young Barley Plants at Different Stages of Maturity*

(*Average of 4 Cultures, 4 Plants to a Culture*)

		14 Days	21 Days	28 Days	35 Days	42 Days	49 Days
Low Nitrate: $\frac{\text{Tops}}{\text{Roots}}$ . . .	Mean.	3.28±.068	4.22±.043	4.34±.063	4.94±.111	4.99±.139	5.33±.082
	Diff. .		.94±.080	.12±.076	.60±.127	.05±.177	.34±.161
Medium Nitrate: $\frac{\text{Tops}}{\text{Roots}}$ . . .	Mean.	3.32±.095	4.34±.088	5.13±.076	6.56±.102	7.71±.084	8.28±.135
	Diff. .		1.02±.129	.79±.116	1.43±.127	1.15±.132	.57±.157
High Nitrate: $\frac{\text{Tops}}{\text{Roots}}$ . . .	Mean.	4.13±.129	5.20±.074	5.75±.136	6.87±.083	7.66±.146	9.08±.224
	Diff. .		1.07±.148	.55±.150	1.12±.157	.79±.168	1.42±.267

Experiment II

Behavior of Nitrates when Total Concentrations are Reversed

In the foregoing experiment the three solutions differed in total concentration as well as in the concentration of nitrates. It happened that the solution with the highest nitrate content had also the highest total concentration. The question is naturally raised, then, as to whether or not the increased concentration of the solution rather than the increased nitrate content is responsible for the increased ratio of tops to roots. This question has led to the arranging of a second experiment in order to throw light on this phase of the problem. It is recalled here that Stiles and Miss Brenchley found that the top-root ratios decreased as the concentration was decreased.

For this experiment, Solution II (medium nitrate) remained as in Experiment I. The low-nitrate solution is modified and designated Solution I*a*. In this, the nitrate content is kept the same, but the other salt components are increased to .5 gram per liter, which makes the modified solution about 5 times the original, Solution I. The high-nitrate solution is designated Solution III*a*; it likewise retains the same amount of nitrate as before, but the remaining six salts are reduced to .06 gram each, thereby reducing the total concentration of the solution about one seventh. The composition of the solutions used in Experiment II was as follows:

	Sol. I*a* (Low Nitrate)	Sol. II (Medium Nitrate)	Sol. III*a* (High Nitrate)
KNO_3	.025	.25	.5
KH_2PO_4	.5	.1	.06
K_2SO_4	.5	.1	.06
$Ca(NO_3)_2$	.025	.25	.5
$Ca(H_2PO_4)_2$	.5	.1	.06
$CaSO_4$	.5	.1	.06
$Mg(NO_3)_2$	.025	.25	.5
$Mg(PO_4)_2$	.5	.1	.06
$MgSO_4$	.5	.1	.06
Total	3.075	1.35	1.86

The highest concentration here is somewhat above that of Sachs' solution mentioned above. The newly made solutions had the following pH values: Sol. I*a*, 5.59; Sol. II, 6.24; Sol. III*a*, 6.24. Iron was supplied as in Experiment I.

Twenty cultures were set up for each solution in the same manner as previously described. Four of these, constituting a series, were harvested every two weeks. The solutions were changed weekly, but were filled up every day to make up for water lost. The experiment began October 28, 1920, and ran for 56 days. Dry weights only are recorded.

Weather conditions throughout the duration of this experiment were not favorable for plant growth; the continual cloudiness together with the late autumn season made for much less growth in this set of experiments than occurred in Experiment I. These conditions are taken to account for the unsteadiness of growth which is shown from a study of the actual dry-weight values, as well as from a study of the magnitude of the probable errors of the mean ratios of tops to roots which have been computed in each case. The results of the four series of Experiment II are given in table 5.

It will be remembered that the low-nitrate solution now contains 3,075 p.p.m.; the medium-nitrate, 1,350 p.p.m., and the high-nitrate, 1,860 p.p.m. The ratios of tops to roots do not appear to be affected appreciably by the concentrations of the solutions; for in this experiment we have not simply the reverse conditions as to concentration, but the total concentration of the low-nitrate solution is now nearly 5 times its former value, yet results obtained throughout the four series are uniformly comparable to those obtained in Experiment I. The significance of the data obtained in this experiment is clearly shown from a study of the differences of the means of the ratios of the different solutions, taking into consideration the probable errors (table 5). Concentration as such, therefore, has shown no effect upon the ratio of tops to roots.

Growth Features

Root growth of cultures in Solution I*a* (3,075 p.p.m.) was of a different character from that in the two other, less concentrated solutions. The roots are much shorter, more turgid, and the individual fibers are greater in diameter. Data for length of tops and roots taken for the first three series are as follows:

		Sol. I*a* (Low Nitrate)	Sol. II (Medium Nitrate)	Sol. III*a* (High Nitrate)
Series 1	Tops	15.2 cm.	18.3 cm.	16.2 cm.
	Roots	7.75 “	16.0 “	16.2 “
Series 2	Tops	37.88 “	34.25 “	34.5 “
	Roots	9.28 “	18.5 “	19.5 “
Series 3	Tops	40.9 “	39.5 “	41.6 “
	Roots	11.1 “	19.1 “	17.0 “

The length of tops continues about the same during these three series. No differences were observed as to health and vigor of these cultures, so the effect of this more highly concentrated solution seems to be shown in bringing about a change in the morphological character of the root. Since the growth values in this solution relative to the medium-nitrate solution (which is the same as that used in Experiment I, both as to nitrate concentration and as to total concentration) and in Solution III*a* are practically the same as they were where we had the reverse concentration conditions, it would seem that the physiological processes underlying this change are of a different order and, being within the root itself, do not affect the ratio of tops to roots. Another fact indicated is that there is probably a critical concentration of solution beyond which the root will respond morphologically more or less sharply; evidence for this is shown from the data that the longest roots were in the high-nitrate solution in Experiment I, which had a concentration of 2,100 p.p.m., while with a concentration of 3,075 p.p.m. they are shortest and are otherwise different in form.

Increase in Ratio of Tops to Roots at Different Stages of Maturity in Young Plants

Table 5*a* shows the increase in the ratio of tops to roots, at different stages of maturity as the plants grew under the conditions of this experiment. This table shows results similar to those secured in Experiment I.

TABLE 5. *Experiment II (Barley); Behavior of Nitrates when Total Concentration is Reversed (Dry Weights in Grams)*

	Sol. I*a* (Low Nitrate)			Sol. II (Medium Nitrate)			Sol. III*a* (High Nitrate)		
	Tops	Roots	Tops/Roots	Tops	Roots	Tops/Roots	Tops	Roots	Tops/Roots
Series 1, *14 days*	.0437	.0135	3.23	.0883	.0200	4.41	.0632	.0163	3.94
	.0559	.0183	3.05	.0758	.0188	4.03	.0654	.0146	4.47
	.0587	.0182	3.23	.0740	.0178	4.15	.0624	.0160	3.90
	.0650	.0182	3.57	.0710	.0164	4.34	.0624	.0150	4.16
Total ..	.2233	.0682		.3091	.0730		.2534	.0619	
Mean ..			3.27 ± .073			4.32 ± .058			4.12 ± .088
Difference	High Nitrate − Low Nitrate = .85 ± .114.								
Series 2, *28 days*	.1500	.0313	4.19	.1841	.0322	5.72	.1867	.0290	6.44
	.1430	.0263	5.43	.2040	.0320	6.38	.1792	.0270	6.64
	.1733	.0346	5.00	.1643	.0263	6.21	.2085	.0295	7.07
	.1462	.0340	4.30	.2122	.0329	6.45	.1795	.0290	6.23
Total ..	.6123	.1262		.7646	.1244		.7539	.1145	
Mean ..			4.88 ± .157			6.19 ± .111			6.59 ± .122
Difference	High Nitrate − Low Nitrate = 1.71 ± .199.								
Series 3, *40 days*	.3310	.0707	4.70	.3370	.0478	7.05	.4020	.0526	7.64
	.2586	.0577	4.48	.4529	.0651	6.96	.4616	.0525	8.79
	.2810	.0600	4.68	.4917	.0715	6.88	.4213	.0562	7.49
	.3308	.0600	5.51	.3938	.0622	6.33	.5308	.0671	7.91
Total ..	1.2014	.2484		1.6754	.2466		1.8157	.2284	
Mean ..			4.84 ± .153			6.81 ± .108			7.95 ± .122
Difference	High Nitrate − Low Nitrate = 3.11 ± .195.								

TABLE 5—*Continued.*

Series 4, *56 days*	.7350	.1257	5.84	1.3527	.1334	10.14	1.3164	.1611	8.11
	1.0361	.1571	6.59	1.3000	.1280	10.15	1.3200	.1350	9.77
	.5730	.0943	6.07	1.1826	.1418	8.34	1.2264	.1172	10.46
	.9527	.1772	5.38	1.1953	.1600	7.47	1.2432	.1238	10.04
Total ..	3.2968	.5543		5.0306	.5632		5.1160	.5371	
Mean..			5.97±.169			9.03±.454			9.59±.347

Difference........................High Nitrate − Low Nitrate = 3.62 ± .386.

TABLE 5*a*. *Experiment II; Increase in Ratio of Tops to Roots in Young Barley Plants at Different Stages of Maturity*

(*Averages of 4 Cultures, 4 Plants to a Culture*)

		14 Days	28 Days	40 Days	56 Days
Low Nitrate:					
$\frac{\text{Tops}}{\text{Roots}}$..........	Mean.........	3.27±.073	4.88±.157	4.84±.153	5.97±.169
	Diff...........		1.61±.173		1.13±.228
Medium Nitrate:					
$\frac{\text{Tops}}{\text{Roots}}$..........	Mean.........	4.23±.05	6.19±.111	6.81±.108	9.03±.454
	Diff...........		1.96±.125	.62±.154	2.22±.466
High Nitrate:					
$\frac{\text{Tops}}{\text{Roots}}$..........	Mean.........	4.12±.088	6.59±.122	7.95±.122	9.59±.347
	Diff...........		2.47±.150	1.36±.172	1.64±.368

Experiment III

Effect of Nitrates upon Ratio of Tops to Roots in Corn Plants

In order to get comparative data as to the effects of nitrates, Experiment III was conducted with corn. A selected variety of field corn was used. The method was practically the same as that used for barley. Three corn seedlings were employed to a culture and three cultures constituted a series. The first series was removed at the end of 27 days, the second at the end of 34 days, and the third at the end of 41 days. Both green and dry weights of tops and roots are recorded, as are also the probable errors of the means of the dry-weight ratios. It was found practicable in this experiment to use two solutions only, the low-nitrate and the medium-nitrate solutions.

The results are given in table 6. The difference between the ratios of tops to roots is not significantly large for the first series; this was true also of the first series with barley, and indicates that the seedling is influenced more greatly by the composition of the solutions after the tops have begun to function actively. The second series, however, gives a difference in favor of the medium-nitrate solution of 1.88 ± .261, and the third series a difference of 3.34 ± .678, as will be seen from the table.

The increase in ratio of tops to roots as the plants grow older is brought out in this experiment, as was the case for barley in Experiment I. Thus the results of Experiment III confirm in every way those obtained with barley. The three experiments leave no doubt as to the effect of nitrates in increasing the ratio of tops to roots in these two plants under the conditions employed.

It is worthy of note, also, that there is little difference in the efficiencies of these two solutions as measured by total growth produced either in green or in dry weight.

			(Green Wt.)	(Dry Wt.)
In Series 1,	Sol. I,	Total Growth	39.3199	3.8014 (low nitrate)
	Sol. II,	" "	36.9930	3.2390 (medium nitrate)
In Series 2,	Sol. I,	" "	49.4250	4.8440
	Sol. II,	" "	54.6613	4.4670
In Series 3,	Sol. I,	" "	61.4600	5.7998
	Sol. II,	" "	61.5000	5.5905

These figures show that plants in the higher-nitrate solution have relatively higher water contents and correspondingly smaller dry weights. In the light of this fact, there is great need of more careful consideration than is usually given as to what one desires of the plant before concluding as to the effectiveness of a particular solution or a particular fertilizer; also, dry-weight determinations which are not checked up occasionally with the green weights may be wholly misleading as to the actual amount of growth which may have taken place during a given period.

Growth Features

This was not a very favorable season for growing corn (February 1–March 13), hence growth was slow, though the plants showed no external evidence either in tops or in roots of not being entirely healthy. No particular differences were noticeable in height or color of tops, but the roots in the low-nitrate solution were notably longer and larger from the first series (table 6).

Experiment IV

Effect of Increasing the Concentration of Nitrates upon the Ratio of Tops to Roots in Flax

In the course of preliminary experiments, it was noticed that the ratios of tops to roots in certain plants were not appreciably altered by increasing the nitrate concentration of the solution. One of these plants was flax. Experiment IV was conducted, therefore, with flax to get more data on this point. A seed type of flax was used, one which had been selfed for several generations, thus assuring a certain grade of stability.

The plants grew 41 days in the greenhouse simultaneously with the corn in Experiment III. They were green and healthy throughout, but

Table 6. *Experiment III (Corn); Ratio of Tops to Roots as Nitrate Concentration is Increased (Green and Dry Weights in Grams)*

		Series 1—27 Days			Series 2—34 Days			Series 3—41 Days		
		Tops	Roots	Tops/Roots	Tops	Roots	Tops/Roots	Tops	Roots	Tops/Roots
Sol. I (Low Nitrate)	Green Wt...	8.8069	2.6230		11.1420	2.482		17.7100	3.9100	
		10.6200	3.9400		14.3820	3.9250		17.4800	4.8300	
		10.8150	2.5150		14.6120	2.6820		14.4400	3.0900	
	Total.......	(30.2419)	(9.0780)		40.3360	9.0890		49.6300	11.8300	–
	Dry Wt.....	.8760	.2033	4.31	1.1300	.2000	5.65	1.3416	.2511	5.34
		1.1141	.2900	3.84	1.4760	.2840	5.20	1.9318	.3000	6.43
		1.1060	.2120	5.21	1.5130	.2410	6.27	1.7420	.2333	7.46
	Total......	(3.0961)	(.7053)		4.1190	.7250		5.0154	.7844	
	Mean......			4.44±.261			5.71±.211			6.41±.407
Sol. II (Med. Nitrate)	Green Wt...	10.7390	2.1548		16.9420	1.7134		19.1500	2.9000	
		9.8600	2.4342		16.6519	2.3700		15.1000	2.4400	
		10.0550	1.7600		14.9600	2.0240		19.0000	2.9100	
	Total......	(30.6540)	6.3490		(48.5539)	(6.1074)		53.2500	8.2500	–
	Dry Wt.....	.9132	.1900	4.81	1.3900	.1800	7.72	1.9316	.1662	11.63
		.8670	.1864	4.65	1.3060	.1650	7.91	1.4852	.1611	9.22
		.9239	.1795	5.15	1.2520	.1740	7.14	1.5964	.1900	8.40
	Total.......	(2.7041)	(.5559)		(3.9480)	(.5190)		(5.0132)	.5173	
	Mean.......			4.87±.099			7.59±.056			9.75±.675

Difference........................Medium N. – Low N. = 1.88 ± .261. Medium N. – Low N. = 3.34 ± .678.

those in the low-nitrate solution seemed slightly more vigorous and had slightly longer tops for each series, as well as longer roots. The lengths of tops and roots are given for two series:

		Sol. I (Low N.)	Sol. II (Med. N.)	Sol. III (High N.)
Series 2 (36 days)	Tops	19 cm.	17.5 cm.	17.5 cm.
	Roots	26.3 "	20 "	18.3 "
Series 3 (41 days)	Tops	21.3 "	19.6 "	19.3 "
	Roots	28.6 "	26.5 "	18.5 "

Table 7 gives the green weights and dry weights obtained in this experiment. It will be noticed that this plant makes no appreciable response, in the ratio of tops to roots, to increased nitrate content of the solution. In other words, it would seem that in the case of flax we are dealing with a plant of an entirely different physiological constitution in so far as its ability to utilize nitrates is concerned. To what range it might respond, the limits of this experiment have not permitted investigation, but this fact presents a very interesting problem from the standpoint of flax nutrition (table 7).

Studies to Determine the Direct Effect of Nitrates upon Root Growth by Growing Roots of Certain Plants in Pure Cultures

Data submitted in the foregoing experiments show conclusively that concentration of nitrate ions in the nutrient solutions used, regardless of the total salt concentration of the solution, was the determining factor in altering the ratios of top growth to root growth in barley and corn. These experiments do not give evidence, however, as to how this alteration comes about. The writer has, therefore, carried out another set of experiments with the hope of finding out whether nitrates check the growth of roots directly, or whether the greater ratio of tops to roots as well as the actual reduction in root growth in the higher-nitrate solution may be due chiefly to processes set up in the tops which affect the distribution of food between tops and roots.

Several methods were followed in this phase of the work. The effort was centered chiefly in removing the influence of tops and noting the effect of different treatments upon roots.

For these experiments, the same high-, medium-, and low-nitrate mineral nutrient solutions were employed as in Experiment I, but to each solution glucose was added at the rate of 2 grams per 100 cc. of solution, to supply the carbohydrate which normally comes from the leaves.

The cultures were grown singly in 250-cc. globe flasks, or in some cases in Erlenmeyer flasks, in 100 cc. of solution. The cleaned culture flasks

TABLE 7. *Experiment IV (Flax); Ratio of Tops to Roots as Nitrate Concentration is Increased (Green Wt. and Dry Wt. in Grams)*

		Series 1—21 Days			Series 2—36 Days			Series 3—41 Days		
		Tops	Roots	Tops/Roots	Tops	Roots	Tops/Roots	Tops	Roots	Tops/Roots
Sol. I (Low Nitrate)	Green Wt.	0.8737	.3546		4.4100	1.5100		4.8100	1.2700	
		1.0359	.5120		4.2600	1.5100		4.9350	1.2300	
		0.7751	.3400		4.6400	1.5850		5.9150	1.5400	
	Total	2.6847	1.2066		13.3100	4.6050		15.6600	4.0400	
	Dry Wt.	.1173	.0433		.4940	.1210		.6800	.1580	
		.1283	.0476		.4840	.1130		.6775	.1700	
		.1055	.0388		.5340	.1200		.8210	.1878	
	Total	.3511	.1297		1.5120	.3540		2.1785	.5158	
	Mean			2.7			4.2			4.2
Sol. II (Med. Nitrate)	Green Wt.	.5140	.2122		3.5700	1.4100		4.9150	1.3800	
		.6960	.2347		3.2000	.9800		4.8978	1.4830	
		.6326	.1421		3.5300	1.1750		4.3900	1.3916	
	Total	1.9026	.5890		10.2400	3.5650		14.2028	4.25466	
	Dry Wt.	.0600	.0245		.3850	.0940		.6770	.1740	
		.0845	.0335		.3520	.0830		.6520	.1654	
		.0900	.0358		.4130	.1000		.5810	.1533	
	Total	.2345	.0938		1.1500	.2770		1.9100	.4927	
	Mean			2.5			4.18			3.87
Sol. III (High Nitrate)	Green Wt.	.4614	.0768		3.5000	1.2100		5.0500	1.7450	
		.6041	.1000		3.1520	1.0900		5.7168	1.7750	
		.5379	.0890		3.5700	1.1300		4.3000	1.3060	
	Total	1.6034	.2658		10.2220	3.4300		15.0668	4.8260	
	Dry Wt.	.0559	.0220		.4200	.0950		.6869	.1649	
		.0715	.0300		.3900	.0980		.7668	.1659	
		.0674	.0266		.4350	.1040		.5725	.1416	
	Total	.1948	.0786		1.2450	.2970		2.0262	.4724	
	Mean			2.5			4.2			4.29

containing the solutions were plugged with cotton and sterilized for 30 minutes at 15-pounds' steam pressure.

Seeds

The seeds for the cultures were carefully selected on the basis of their vigor and uniformity. They were first sterilized for 4 hours by the calcium hypochlorite method developed by Wilson (34) in 1915, and after being washed with sterile water they were transferred for germination to sterile agar in plugged 50-cc. vials. A word might be said here with reference to the Wilson solution as a sterilizing medium. A claim for this method which would highly recommend it is that seeds may be effectively transferred from it directly to the germinating medium without washing in sterile water. When one is dealing with individually weighed and recorded seeds, the percentage germination of these seeds becomes a very important factor both from the standpoint of accuracy of results and from that of labor involved. It is the writer's experience that unless the grains of corn are washed thoroughly on transference from the calcium-hypochlorite solution to an agar germinating medium, the percentage of germination is greatly reduced, sometimes as much as 60 percent.

Cultures were grown in the greenhouse in a dark chamber specially constructed to allow for the free circulation of air.

In Experiment V, the tips only of corn roots were employed. When the radicle had reached a length of 2 to 3 cm., it was removed with a sharp scalpel, under sterile conditions, dropped into the sterile culture solution, and set away in the dark chamber for growth.

The results of two series of 20 and 44 days' growth respectively are presented in table 8. A third series was conducted, but as several cultures in the low- and medium-nitrate solutions became contaminated, comparative results are not possible, though the results in the high-nitrate solution both as to green and as to dry weight are essentially the same as those reported in Series 1. Eight cultures constituted a series.

The results of Series 1 (Experiment V), in which root tips only were used, show clearly, as far as green-weight determinations are concerned, that there is an increase in weight when the amount of nitrates in the solution is increased. The medium-nitrate solution appears slightly more efficient here, as was the case with cultures of entire plants growing in the mineral-nutrient solutions alone.

The dry-weight determinations for the twenty-day period do not, at first glance, appear to give such positive evidence as those of green weight of an increased growth as the nitrate content of the nutrient solution is increased, since there are such small differences among the weights for this period; but the fact has already been demonstrated and pointed out that dry-weight determinations are by no means safe criteria of growth of plants

TABLE 8. *Experiment V; Effect of Increasing Nitrates on Growth of Root Tips of Corn (Dry Weights in Grams)*

	Sol. I (Low Nitrate), Roots			Sol. II (Medium Nitrate), Roots			Sol. III (High Nitrate), Roots		
	Green Wt.	Dry Wt.	Length	Green Wt.	Dry Wt.	Length	Green Wt.	Dry Wt.	Length
Series 1, *20 days*...	.1100	.0069		.1158	.0100		.1172	.0077	
	.0895	.0080		.2006	.0130		.1334	.0076	
	.1769	.0214		.0852	.0060		.1071	.0059	
	.2153	.0196		.2217	.0077		.1493	.0124	
	.1202	.0096		.1252	.0157		.1613	.0126	
	.1576	.0142		.1515	.0100		.1775	.0102	
	.1777	.0062		.2108	.0125		.1592	.0130	
	.1625	.0082		.1331	.0128		.1248	.0089	
Mean...........	.1262±.0124	.0118±.0014	18.8 cm.	.1555±.0119	.0110±.00075	18.6 cm.	.1475±.0057	.0098±.00064	21.2 cm.
	Dry Wt.			Dry Wt.			Dry Wt.		
Series 2, *44 days* ..	.0202		43.2	.0166		40.5	.0045		26.5
	.0255		50.0	.0326		41.2	.0104		37.0
	.0109		27.0	.0251		54.0	.0131		44.5
	.0116		20.0	.0231		30.0	.0051		21.5
	.0134		28.0	.0082		27.0	.0094		33.0
	.0119		25.5	.0067		22.5	.0047		20.5
	.0141		16.0	.0086		31.5	.0089		22.0
	.0097		28.5	.0087		30.5	.0135		47.5
Mean...........	.01466		28.5 cm.	.0162		34.65 cm.	.0087		31.5 cm.

when grown under different environmental conditions. Those plants which had a higher nitrate substrate for growth had a relatively smaller dry-matter content, while the reverse is true with those having less nitrates available. This is clearly brought out in the figures under consideration (table 8), and more clearly still in table 10. It would appear that metabolic processes such as respiration—not accompanied by increase in dry matter, but accompanied by an accumulation of aqueous or other volatile material—are accelerated in consequence of the larger available supply of nitrates. Physically, plants growing under such conditions take on the character of greater succulency.

The green weights were not determined for the 44-day-period cultures, but the dry weights are essentially as in Series 1, with a relatively higher dry-matter content for the low-nitrate cultures and a strong indication of an increased utilization of food in respiratory processes in the cultures in the high-nitrate solution.

Measurements were made of length of roots for the 20- and 44-day periods respectively in order to determine whether or not the differences in nitrate content of the solutions had any stimulating effect on length. While the average lineal growth is somewhat higher in solutions with larger nitrogen content, the difference is not large enough to be significant (table 8). It is interesting, however, to note that these root tips have continued to grow through a period of 44 days, increasing considerably in length, as is shown in the table, but showing little or no increase in total dry matter.

Experiment VI

Effect of Increased Nitrates upon Growth in Length of Isolated Tips of Radish Roots

Germination of the seeds and preparation of the root tips for these cultures were carried out in the same manner as that described for corn. The cultures ran for 15 days.

The results are given in table 9. They show no striking response in length of root of this plant to the increased nitrate content of the solution.

TABLE 9. *Radish; Results of 15 Days' Growth (Lengths in Centimeters)*

Culture	Solution I (Low N.)	Solution II (Medium N.)	Solution III (High N.)
1	9	12	9.5
2	7	7.3	6.3
3	5	20.5	12.5
4	7.5	13.5	8
5	11	6	8
Mean	8	11.8	8.8

Experiment VII

Effect of Increased Nitrates upon Growth of Roots of Corn with Plumule and Endosperm Removed, but with Cotyledon Attached

The cultures for this experiment were set up in exactly the same manner as that described for Experiment V, with the exception that the corn roots used had the cotyledon attached. The plumule and the endosperm were both removed under sterile conditions. Both green and dry weights are recorded in table 10. Two series were conducted, with 8 individual cultures to a series. Each series grew for twenty days. At the end of the growth period the remains of the cotyledon were removed, when the green and dry weights were determined.

A study of the two series shows an unmistakable increase in growth in the roots grown in solutions with higher nitrate content. This is more obvious in the green weights than in the dry weights. It is to be expected that the dry weight would be relatively less in the high-nitrate solution, for the more vigorous growth would be attended by more active respiratory processes which would consume much of the food that would otherwise be left for building material. Comment upon this table is hardly necessary. The probable errors of the differences, while apparently not so significant for the dry weights, are clearly so for the green weights. The greater succulency, as has already been mentioned, of the plants growing in the higher-nitrate solutions is a factor to be considered carefully in connection with the differences in dry weight between these and the lower-nitrate cultures.

Several series of experiments were carried out by growing plants in mineral nutrients both in the light and in the dark chamber, with plumules removed, but the results were wholly negative as to any effects of nitrates directly upon the roots.

Discussion

The experiments reported in this paper show clearly that increasing the nitrate concentration of a nutrient solution, within the range investigated, will increase the ratio of top growth to root growth in certain plants. In some cases this alteration of ratio is attended with very little difference in the total yield of the different solutions used.

In the literature, attention has been given to some extent to the effect of differences in total concentration upon the ratio of tops to roots, but no work of controlled nature has been done hitherto to detect and to localize the cause of specific effects of particular mineral nutrients on this ratio.

The results of Experiments I and II bring out the fact that concentration as such is without effect, but that the composition of the solution is the factor of most importance. This work confirms the conclusions of Cameron (5), Hoagland (15), Shive (28), and Duggar (9) with respect to placing

TABLE 10. *Experiment VII; Effect of Increasing Nitrates on Growth of Root Tips of Corn (Green and Dry Weights in Grams)*

	Sol. I (Low Nitrate), Roots		Sol. II (Medium Nitrate), Roots		Sol. III (High Nitrate), Roots	
	Green Wt.	Dry Wt.	Green Wt.	Dry Wt.	Green Wt.	Dry Wt.
Series 1, *20 days*	.5187	.0905	1.5181	.1749	.8229	.0875
	.3502	.0926	.8762	.0950	.7495	.0663
	.6724	.1116	1.2913	.1199	.7012	.0634
	.4435	.0766	1.1171	.1238	.5088	.0640
	.6469	.1054	.5605	.0783	1.1619	.1180
	.3624	.0673	1.3884	.1459	2.1832	.1765
	.2209	.0448	.4510	.0548	1.8442	.1584
	.2100	.0390	.5336	.0812	1.0061	.0968
Mean	.4369±.0413	.0785±.0064	.9670±.0890	.1092±.0093	1.1222±.1403	.1039±.0104

Difference......Medium N. − Low N. = .0307 ± .0113 (Dry Wt.). (Green Wt.) High N. − Low N. = .6853 ± .145.

Series 2, *20 days*	.7805	.0883	2.1530	.1853	2.0676	.1469
	.4583	.0667	1.4627	.1442	1.5892	.1213
	.7412	.0953	1.0294	.1088	1.4977	.1260
	.7340	.0900	.2330	.0396	.6623	.0568
	.5809	.0586	.3019	.0492	.7305	.0871
	.5020	.0734	.5391	.0680	1.3100	.1534
	.3729	.0549	1.2484	.1253	1.1824	.1124
	.4674	.0714	.5818	.0490	1.4398	.1102
Mean	.5799±.0367	.0747±.0036	.9437±.157	.0962±.0126	1.30996±.0346	.11426±.0074

Difference............(G. Wt.) High N. − Low N. = .7301 ± .0504. (Dry Wt.) High N. − Low N. = .0396 ± .0082.
(G. Wt.) High N. − Medium N. = .3663 ± .161.

importance upon composition of the nutrient solution. It would seem that when great emphasis is placed upon concentration as the cause in altering the ratio of tops to roots, as has been done by certain English workers in this field, greater care should be taken in checking up solutions used in order to make sure that there are sufficient mineral nutrients present at all times in the solution, for we cannot avoid the conclusion that one or more ions may become limiting factors in these more dilute solutions. Hoagland has shown that NO_3, PO_4, and K may be exhausted if the solution is run long enough without renewal. Starvation and concentration effects may be entirely different, yet their outward manifestation may often be indistinguishable.

The hydrogen-ion concentration was carefully checked for each set of experiments. The pH value ranged from 5.59 to 6.47. Though the low-nitrate solution had the highest hydrogen-ion concentration when it had also the highest total concentration (3,075 p.p.m.), this hydrogen-ion concentration could not be a factor in determining the ratio of tops to roots of plants growing in it, since the ratio is of the same relative order in respect to those of medium-nitrate and high-nitrate solutions as it was when the hydrogen-ion concentrations of the three solutions had the same value.

Proof is brought forth in the pure-culture experiments that nitrates have no direct checking effect upon roots, but that, on the other hand, the growth of roots is increased directly with the nitrate content of the solution, provided the disturbing influence of tops is not at hand. Thus, since there is no direct checking action of nitrates upon root growth, we shall have to explain the increased ratio of tops to roots on some other basis. Miss Brenchley (2) has offered an explanation of this phenomenon as follows:

> It seems as though the plant makes every endeavor to supply itself with adequate nutriment, as if, when the food supply is low, it strives to make as much root growth as possible so as to offer the greatest absorbing surface for whatever nutriment may be available.

Realizing that this explanation was not adequate, she has later offered a more elaborate explanation (3). Both explanations are purely teleological and do not give the plant physiologist any satisfaction as to the mechanism by which the tops in plants come to make such increase over roots.

Livingston's (17) explanation, which is based upon his experiments with plants growing in poor soil, is that the poor soil brings about such morphological changes in the root as to render it incapable of absorbing water, and thus, that the growth of tops is limited because of an inadequacy of water.

Curtis (36) has suggested that the effect of nutrients, especially of nitrates and phosphates, upon root growth might result, not from any direct action on or in the roots, but from an indirect effect caused by a change in the supply of food available for roots through the effect of the nutrient upon the use of the food by the tops.

In the experiments studied here, we find the increase of ratio of tops to roots to be due to nitrate ions. It has been shown, further, that nitrates do not retard the growth of roots directly, but that, on the other hand, the root increases in growth in the presence of nitrates if carbohydrates are also present. This fact shows that carbohydrates may be a limiting factor.

Further evidence along this line is shown in some unpublished data of Mr. W. C. Muenscher of the department of botany of Cornell University. Using averages of large numbers of water cultures (12 in one case, 25 in another), he found that the ratio of dry weight of tops to roots (barley) was always greater for plants grown in the shade than for those in the unobstructed sunlight. A brief summary of his data (in dry weights) is as follows:

	Sunshine	Shade	Time of Year
(1) Ratio, $\frac{\text{Tops}}{\text{Roots}}$	5.10	7.84	Jan. 19 to Feb. 24
(2) Ratio, $\frac{\text{Tops}}{\text{Roots}}$	4.26	5.96	Aug. 4 to Sept. 8.

This table shows also that the ratio of tops to roots was higher for plants grown in winter months than for those grown in the summer-fall season. Working as he was with a full nutrient solution, the production of carbohydrates seems to be the limiting factor, which accounts for the difference under the two conditions. Since the amount of carbohydrates produced in the shade or during the season of relatively less sunshine would be relatively less than that produced in greater light, the tops in the shade or in the reduced light of winter would use relatively more of that amount, thus leaving less for the roots.

Results from the pruning experiments of Chandler (6) may also have bearing on this point. He has shown that, while pruning is a dwarfing process, it checks root growth more than it does top growth. It is possible that, in removing a large leaf surface which is not compensated for by new growth, the carbohydrate production is cut down, and, as the increased vigor of the remaining shoots following pruning tends to use the carbohydrates manufactured, there is a smaller amount to be carried to the roots.

In the case of nitrates, it is likely that, when the concentration of nitrates is increased, relatively more of the manufactured carbohydrates tend to be consumed in the growth processes of the tops. Little is left for distribution to the roots. The roots, therefore, will not make the same relative growth as tops, and their growth may be severely checked in this way. This will explain also the sparse development of aërial roots in the high-nitrate cultures.

Plants like flax which show no increase in ratio of tops to roots as the nitrate content in the solution is increased may not be able to absorb nitrates beyond a certain amount or may not be able to utilize them beyond a certain amount, which is adequate in the low-nitrate solution; thus nitrates do not seem to become the limiting factor at the concentrations used.

Summary and Conclusions

1. Very few researches have been carried on to determine the factors which affect the ratio of top growth to root growth in plants. A solution of this problem is of interest both to the plant physiologist and to the practical plant grower.

2. The purpose of the present study is to determine more exactly how increasing the nitrate content of a nutrient solution that is otherwise adequate for plant growth will affect the actual top and root growth as well as the ratios of top to root growth; and further to suggest an explanation of the mechanism of the results obtained.

3. Definite effects of particular mineral nutrients, under controlled conditions, upon top-root ratios, have been given little attention, though work has been reported as to the effect of poor soil and of a decrease in concentration of the nutrient solution.

4. It has not been satisfactorily demonstrated that decreased concentration as such will decrease the ratio of tops to roots in plants; on the other hand, it has been demonstrated in the present set of experiments that decreased concentration is ineffective in contrast with nitrate ions.

5. A combination of nutrient salts is devised and used whereby nitrate ions may be increased or diminished widely without disturbing very much the other components of the solution.

6. In all cases, barley and corn show significant increases in ratio of tops to roots as nitrate concentration of the solution is increased. This effect of nitrates was shown to be independent of the total concentration of the solution, and independent of the hydrogen-ion concentration.

7. Flax grown for 41 days did not show an increase in ratio of tops to roots as nitrate content of the solution was increased, nor was there an increase with age after 21 days.

8. The efficiencies of the three solutions, as judged by total dry weights of tops and roots, were not greatly different.

9. Roots were grown in pure cultures with a supply of sugar. Some had plumules only removed; others had plumules and endosperms removed; in the third set, only the tips of the roots (2 cm. to 3 cm.) were employed. The results showed clearly that nitrates do not check, directly, root growth; but they increased the growth of roots in all cases.

10. The fact is brought out that neither dry weight nor length of root is a safe measure of growth in plants.

11. Evidence has been given in these experiments that roots grown in pure cultures, in absence of light, are able to utilize the nitrates and carbohydrates present in the solution for growth and respiratory processes.

12. Conclusion: The increased ratio of tops to roots which results from increasing the amount of nitrate in the solution may be explained on the basis of increased use of carbohydrates in the tops because the greater nitrogen supply makes for greater growth. This results in a decrease in the supply of carbohydrates for the roots, which may bring about an absolute or a relative reduction of root growth.

This study was carried out in the laboratory of plant physiology at Cornell University, and the writer is deeply indebted to Professor O. F. Curtis, who suggested the problem, for valuable and helpful criticisms in the course of the work. The writer also owes thanks to Professor H. H. Love of the plant-breeding department, who furnished the pure line of barley used, and to Professor C. H. Hutchinson of the same department for the pure line flax seeds.

REFERENCES

1. **Birner, H., und Lucanus, B.** Wasserculturversuche mit Hafe. Agric-chem. Versuchsstat. zu Regenwalde **8** : 128–177. 1866.
2. **Brenchley, W. E.** Effect of concentration of nutrient solution on growth of barley and wheat in water cultures. Annals Bot. **30** : 77–90. 1916.
3. ——. On the relations between growth and environmental conditions of temperature and bright sunshine. Ann. Appl. Biol. **6** : 211–245. 1919–1920.
4. **Brezeals, J. F.** Effect of concentration of nutrient solution upon wheat cultures. Sci. n. ser. **22** : 146–149. 1905.
5. **Cameron, F. K.** The soil solution. Easton, Pa., 1911.
6. **Chandler, W. H.** Some results as to response of fruit trees to pruning. Proc. Amer. Soc. Hort. Sci. **16** : 88–101. 1919.
7. **Coupin, H.** Sur l'absorption des sels minéraux par le sommet de la racine. Compt. Rend. Acad. Sci. Paris **169** : 242–245. 1919.
8. **Dassonville, C.** Influence des sels minéraux sur la forme et la structure des végétaux. Rev. Gén. Bot. **10** : 15–25, 370–380. 1898.
9. **Duggar, B. M.** Hydrogen ion concentration and the composition of the nutrient solution in relation to the growth of seed plants. Ann. Mo. Bot. Gard. **7** : 1–49. 1920.
10. **Du Sablon, Leclerc.** Traité de physiologie végétale et agricole. Pp. 218–222, 234–235. Paris, 1911.
11. **Gile, P. L., and Carero, J. O.** Assimilation of iron by rice from certain solutions. Jour. Agr. Res. **7** : 503–528. 1916.
12. **Harris, F. S.** Effect of soil moisture, plant food and age on the ratio of tops to roots in plants. Jour. Amer. Soc. Agron. **6** : 65. 1914.
13. **Hoagland, D. R.** Effect of hydrogen-ion and hydroxyl-ion concentration on the growth of barley. Soil Sci. **3** : 547–560. 1917.
14. ——. Relation of the plant to reaction of the nutrient media. Sci. n. ser. **48** : 422–425. 1918.
15. ——. Relation of the concentration and the reaction of the nutrient medium to the growth and absorption of the plants. Jour. Agr. Res. **18** : 73–117. 1919.
16. **Kraus, E. J., and Kraybill, H. R.** Vegetation and reproduction with special reference to the tomato. Ore. Agr. Exp. Sta. Bull. 149. 1918.
17. **Livingston, B. E.** Relation between root growth and tops. Bot. Gaz. **41** : 139–143. 1906.
18. **Mer, E.** De l'influence du milieu sur la structure des racines. Compt. Rend. Acad. Sci. Paris **88** : 665–668, 1277–1280. 1879.

19. **Moeller, H.** Beiträge zur Kenntniss der Verzwergung. Land. Jahrb. **13** : 167–173. 1884.

20. **Müller-Thurgau, H.** Jahresber. Versuchssta. Wadensweil. **4** : 48–52.

21. **Palladin, W.** Plant physiology (Eng. transl. by B. E. Livingston). Philadelphia, 1918.

22. **Prideau, E. B. R.** Theory and use of indicators. New York, 1917.

23. **Pouget, I., and Chouchak, D.** Influence de la concentration des solutions de substances nutritives sur leur absorption par les végétaux. Compt. Rend. Acad. Sci. Paris **154**: 1709–1711. 1912.

24. ———. Sur la loi du minimum. Compt. Rend. Acad. Sci. Paris **155**: 303–306. 1912.

25. **Saposchnikoff, W.** Bildung und Wanderung der Kohlenhydrate in den Laubblätter. Ber. Deutsch. Bot. Ges. **8**: 233–242. 1890.

26. ——. Eiweissstoffe und Kohlenhydrate der grünen Blätter als Assimilations-producte. (Review) Bot. Centralbl. **63**: 246–251. 1895.

27. **Schimper, A. F. W.** Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora **73**: 207–261. 1890.

28. **Shive, J. W.** A study of physiological balance in nutrient media. Physiol. Res. **1**: 327–397. 1915.

29. **Stiles, W.** Concentration of the nutrient solution and the rate of growth of plants in water cultures. Annals Bot. **29**: 89–96. 1915.

30. ——. Interpretation of results of water culture experiments. Annals Bot. **30**: 327–336. 1916.

31. **Tottingham, W. E.** A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. **1**: 133–245. 1914.

32. **Walster, H. L.** Formative effect of high and low temperatures on growth of barley. Bot. Gaz. **69**: 97–126. 1920.

33. **Wilfarth, H., Römer, H., and Wimmer, G.** Ueber die Nahrstoffaufnahme der Pflanzen in verschiedenen Zeiten ihres Wachstums. Landw. Versuchsstat. **63**: 1–70. 1905.

34. **Wilson, J. K.** Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. **2**: 420–427. 1915.

35. **Zaleski, W.** Ueber die Rolle des Lichtes bei der Eiweissbildung in den Pflanzen. Ber. Deutsch. Bot. Ges. **27**: 56–62. 1909.

36. **Curtis, O. F.** Stimulation of root growth in cuttings by treatment with chemical compounds. Cornell Univ. Agr. Exp. Sta. Mem. 14. 1918.

A PRELIMINARY NOTICE OF GENETICAL STUDIES OF RESISTANCE TO MILDEW IN OENOTHERA[1]

Peter J. Klaphaak and H. H. Bartlett

(Received for publication December 24, 1921)

Introduction

Ever since the senior writer of this paper commenced the growing of Oenothera cultures for experimental purposes, it has been noticed every year that nothing is more characteristic of the various elementary species and hybrids than the great differences that they show in susceptibility to infection by mildew. Thus, among the recently described species, such ones as *Oenothera stenomeres* and *Oe. pratincola* have been uniformly, year after year, heavily infected. Others, such as *Oe. Reynoldsii*, *Oe. numismatica*, and *Oe. scitula*, have been quite as uniformly immune. Similar facts have come to the attention of Professor de Vries, who, in a recent letter, writes that certain of the types grown by him would have been admirably adapted to a study of the inheritance of immunity.

For several years prior to 1919, more or less adequate notes had been kept in the garden as a whole as to the prevalence of mildew, but it became obvious that the solution of the problem would demand special cultures of forms particularly marked in their resistance or susceptibility, as the case might be, which might be handled with the question of disease resistance paramount. Furthermore, since the differences shown by certain pairs of reciprocal hybrids were so astonishingly definite, the one being white with mildew and the other absolutely free, although both were grown in adjoining rows, under identical conditions, and often with interlocking branches, it seemed that the material offered an excellent opportunity for biochemical studies, to be conducted parallel with the genetical work, and designed to trace, if possible, the relationship of immunity and susceptibility to chemical characters of the forms. Consequently, a biochemical study of carefully selected material from these cultures has been undertaken by Mr. Joaquin Mejorada Marañon. His results cannot be reported in this preliminary notice, which, even on the genetical side, aims to present only part of the results, typical of those which are being obtained.

Most of the previous work on the problem of varietal and specific resistance to mildews has been done by Salmon (8, 10). He has published several short papers on varietal susceptibility to the powdery mildew of corn and grasses (*Erysiphe graminis* DC.), and to the hop mildew (*Sphaerotheca Humuli* (DC.) Burr.). He proved the existence of biologic strains among the powdery mildews, especially in his work on *Erysiphe graminis* DC.

---

[1] From the Bureau of Plant Industry, U. S. Department of Agriculture, and the Botanical Garden of the University of Michigan. Published by permission of the Secretary of Agriculture.

That in many cases resistance to mildew is inheritable is without doubt, though little is known of the quality of the resistance or of the genetics of the situation.

No previous work has been reported, as far as we know, on the mildew problem as it is presented by the Oenothera cultures. Atkinson (1, 2) made observations upon immunity and susceptibility to a downy mildew, *Peronospora Arthuri* Farlow, in connection with his genetical studies of *Oenothera pycnocarpa* (susceptible) and *Oe. nutans* (immune). He published his results on the hybrids produced from these crosses, but made only one statement in regard to their susceptibility or immunity to this downy mildew, namely, that the F_1 of the cross *Oe. pycnocarpa* × *Oe. nutans* was susceptible (1). The results, however, suffice to show that the markedly antithetic characters of the Oenotheras, as concerns disease resistance, extend to fungi of other groups than the true mildews with which the present paper is concerned. Of course, the observations of de Vries (12) upon the relative resistance of the mutations of *Oe. Lamarckiana* to infection by Micrococcus are well known, and especially interesting because of the mutational origin of disease susceptibility in the case of mut. *nanella*.

MATERIAL

At the outset, for the sake of clarity, it will be well to state that general observation had indicated that susceptible species when crossed reciprocally with immune ones gave only one immune cross. It was not possible to get immune hybrids by crossing susceptible parents, and in the case of crosses between immune strains, both reciprocals might be immune, or one of them immune and the other susceptible. The results could be formulated in accord with the hypothesis of heterogametism, already set forth in several papers (3, 6). Each species of Oenothera is supposed to produce two types of gametes called α and β gametes. The α gametes are generally female and the β gametes generally male, although other conditions occur, as will be shown later in the discussion of the phenomenon of metacliny. If the immune strains carry a factor **I** for immunity (**i** will then represent the absence of the factor for immunity, or presence of a factor for susceptibility) in only one type of gamete, and if only $\alpha\beta$ combinations are viable, then it can readily be seen that such a strain will breed true for immunity, but will give a susceptible hybrid, one way or the other, when reciprocally crossed with a susceptible strain. If **I** were a dominant factor, all the breeding behavior would be clear, providing it were possible for **I** to be an attribute of the α gamete in some strains, and of the β gamete in others. This hypothesis has been borne out by the results obtained, and the following description of the strains used gives their constitutional formulae as established by the various crosses into which they have entered.

A review of the Oenothera cultures at the Botanical Garden of the University of Michigan in the summer of 1919 led to the selection of the following forms as especially likely to give interesting results:

1. *Oenothera pratincola* Bartlett (4). This species is highly susceptible and was chosen because of the long period (seven years) that it had been grown in self-pollinated lines. Susceptibility of the chosen strain ("Lexington C") had been observed for eight generations. This strain (originally from Kentucky) has, according to the above-mentioned hypothesis of α and β gametes, the genetical constitution $\alpha\mathbf{i}\beta\mathbf{i}$.

2. "*Oenothera biennis Chicago*." This is a hardly distinguishable strain of the preceding, and is referred to under the provisional name assigned to it by de Vries in *Gruppenweise Artbildung* (13). It was chosen because it was essentially identical with the foregoing, but of entirely different provenience. Both forms had been so extensively used in crosses that the opportunity was seized to see if their apparent specific identity would be verified by identical breeding behavior. "*Oe. biennis Chicago*" was received from de Vries in 1912, and had been mildewed every year for seven years. Its genetical constitution has proved to be the same as that of *Oe. pratincola* ("Lexington C"), namely, $\alpha\mathbf{i}\beta\mathbf{i}$.

3. *Oe. mississippiensis* Bartlett (5). This species had been grown for seven seasons under the tentative name "Cartersville," assigned to it by de Vries, who collected it at Cartersville, Mississippi, in 1904, and sent it to one of the writers in 1912. It has always been heavily mildewed. Genetical constitution, $\alpha\mathbf{i}\beta\mathbf{i}$.

4. *Oe. pratincola* hyb. *immunis*. The hybrid which we introduced into our mildew experiments under the name *Oe. pratincola* hyb. *immunis* had an interesting origin. Of the two Oenothera species known from Lexington, Kentucky, and extensively grown in experimental cultures for many years, one, *Oe. pratincola*, is always mildewed, whereas the other, *Oe. numismatica*, is very slightly infected, or not at all. When these species are hybridized, the cross with *Oe. pratincola* as the pistillate parent gives twin hybrids, both of which are mildewed. One of them is strictly like the maternal parent in all characters except one trivial one, namely, the presence of erect, thin-walled, viscid hairs on the flower buds, a character of the paternal parent. This matroclinic cross, known as *Oe. pratincola* hyb. *viscida*, behaves in every respect like *Oe. pratincola*, even to throwing the same mutations. The reciprocal cross, in which *Oe. numismatica* is the pistillate parent, is immune and in all other respects like pure *Oe. numismatica*. According to our hypothesis of α and β gametes we explain these facts as follows:

A. Both the α and β gametes of *Oe. pratincola* are carriers of the factor **i** (susceptibility to mildew).

B. In *Oe. numismatica* the α gamete carries the factor **I** (immunity to mildew), whereas the β gamete carries the factor **i**.

C. The composition of *Oenothera pratincola* hyb. *viscida* is therefore α**i**β**i**, just as is the case in true *Oe. pratincola.*

Now the interesting situation develops. Both true *Oe. pratincola* and hyb. *viscida* have thrown a mutation which closely simulates a certain wild elementary species, namely, *Oe. Reynoldsii* Bartlett (4). However, this mutation coming directly from *Oe. pratincola* is susceptible to mildew, whereas that from hyb. *viscida* is immune. Furthermore, the immunity of the mutation from hyb. *viscida* (called mut. *simulans* because it is indistinguishable from *Oe. Reynoldsii*) is concerned with the β gamete, since the cross *Oe. pratincola* hyb. *viscida* × mut. *simulans* yields an altogether immune hybrid closely resembling *Oe. pratincola* in morphology, but smaller in size, and, perhaps on account of its immunity, very different in coloration. The type comes true from seed, and has been so frequently used in crosses that it has been designated for convenience as *Oe. pratincola* hyb. *immunis.* This hybrid has the composition α**i**β**I**, and, as we interpret the situation, the immunity factor resides in the β gamete by virtue of mutation of the β**i** gamete originally entering into the composition of the line from *Oe. numismatica* to β**I**, this mutation taking place at the time of origin of mut. *simulans.* That the β**i** gamete of *Oe. pratincola* does not undergo such a mutation is shown by the fact that the mutation simulating *Oe. Reynoldsii,* which arises from pure *Oe. pratincola,* and is called mut. *simulans rubricalyx,* because it differs from the otherwise identical mutation from hyb. *viscida* in having red buds, is neither immune itself, nor can its β gamete impart immunity to crosses with the parent type. Thus, *Oe. pratincola* mut. *simulans rubricalyx* is not a type like hyb. *immunis,* but is merely a mildew-susceptible *Oe. pratincola.* It shows neither the immunity nor the small stature of hyb. *immunis,* thus proving that the unique characters of hyb. *immunis* are due to the β gamete from *Oe. numismatica.*

These genetical facts are of no moment to the reader who is interested in the inheritance of the immunity after it has once arisen. They are a necessary part of the present record, however, since hyb. *immunis* has been extensively used in our crosses, being the one available form through which immunity could be transmitted to a cross through the pollen. It should be remarked that hyb. *immunis* breeds quite as true from seed as the other types used as parents. Constitution, α**i**β**I**.

5. *Oenothera cinerescens* Bartlett (5). This species was collected at White Sulphur Springs, West Virginia, in 1912, and has been continuously in culture ever since. It is an outstandingly resistant type, and had been observed to be free from mildew for eight generations up to the time it was used for the crosses described below. Constitution, α**I**β**i**.

The Identity of the Fungus (*Erysiphe Polygoni* DC.)

Salmon (7) has shown that *Erysiphe Polygoni* occurs on a great many different host plants, among which the common garden pea, *Pisum sativum*, is one of the best known. This powdery mildew often bears the name of "mildew of the pea." When the seedling plants of the Oenothera crosses were grown in the greenhouse it was found that the pea mildew did not infect them. The seedling plants were placed in two separate greenhouses, in each about 1,500 plants. In one of the greenhouses there were many pots of garden peas covered with powdery mildew (*Erysiphe Polygoni* DC.). Whenever these plants were moved or shaken, small clouds of spores arose from leaves and stems. None of the Oenothera seedlings standing in the same house showed infection at the time of planting in the field about three months after being placed in the greenhouse. In the other greenhouse a few small plants of *Oenothera nutans* Atkinson & Bartlett, fall seedlings, which were kept during the winter and which were abundantly infected by mildew, were standing near the young seedlings. Before these seedlings were set out in the field, all the susceptible plants among them showed infection. A general exposure to conidia of *Erysiphe Polygoni* from pea did not infect any Oenothera seedlings, while a much less general exposure to conidia of *Erysiphe Polygoni* from *Oenothera nutans* resulted in the infection of a large number. This seems to support the statements of Salmon that, although no distinct morphological differences are found among the strains of *Erysiphe Polygoni* from different host plants, physiological differences may exist, upon which are based the so-called "biologic strains." Searle (11) also proved the existence of biologic strains of *Erysiphe Polygoni* among various hosts.

It was not considered necessary to make any extensive trials at cultivating the Erysiphe of Oenothera on artificial media, since the powdery mildews in general have been amply proved to be obligate parasites by Salmon and others. Salmon (9) found in his experiments that he could grow powdery mildew (oidium) on leaves of *Euonymus japonicus* L. placed on moist filter paper in a damp chamber for as long as 14 days, in which time the leaves were badly affected. In his experiments with biologic strains, Salmon kept his strains growing on living plants.

Examinations were made of prepared slides of leaves from the five different species and strains chosen, in order to determine if any morphological differences might account for the differences in susceptibility and immunity. No such differences were found among the morphological characters of the leaves, suggesting in this case that immunity must have a physiological or chemical basis. Salmon (8) reached the conclusion "*that susceptibility and immunity were due to constitutional (physiological) peculiarities and not to any structural ones.*"

Description of the Mildew, *Erysiphe Polygoni*

A study of the Oenothera mildew showed that in general it conforms to the description of *Erysiphe Polygoni* given by Salmon (7). It is amphigenous; mycelium very variable, persistent to evanescent, thin and effused; perithecia few and scattered, 85–95 μ in diameter, cells distinct, 11–14 μ wide; appendages simple and long, variable in number (3–6), partly interwoven with the mycelium and colorless; asci few (3–5), small and ovate, 50–60 μ × 30–35 μ, containing 3–6 spores, 20–23 μ × 9–12 μ. Conidia (oidium stage) cylindrical to ellipsoid, 33–36 μ × 15–18 μ. The mildew grows very superficially, feeding by means of haustoria extending into the epidermal cells.

Powdery mildew may infect the host plant at any time. Infection in Oenothera is generally first noticed in leaves approaching maturity. Neither very young nor very old leaves will show any infection when a healthy plant is first attacked. At a later stage in the growth the mildew may cover the entire plant. Infections were found as early as April in the greenhouse, and in the fields as soon as the plants were set out. The heaviest infection in the field is commonly found in the summer from June to September, depending upon weather conditions, rainfall being conducive to the spreading of the disease. It is often found that during the summer time susceptible plants are entirely covered with mildew, so as to appear whitish. No evidence has been found that mildew does any great damage to Oenothera plants, as it does to the pea. Though entirely covered by Erysiphe, infected Oenotheras appear to grow normally, to come to bloom and to ripen seeds in exactly the same way, and presumably in the same amount, as healthy plants. Even though highly susceptible, species of Oenothera seem to be very tolerant to the disease.

In our experiments the plants have been under observation during the whole season, and have been classified as immune to powdery mildew if they have shown no infection at any time. It may be objected that in some cases immunity may have been only apparent and due to a position in the field preventing infection. This objection is easily answered. The plants were set out in the field in rows of from 150 to 160 each. Of each of the parent strains chosen, 25 plants were grown to maturity, and of the hybrids about 100 plants. Cultures differing in their susceptibility to mildew were grown near together, so that in many cases an immune strain or species was grown among highly susceptible strains, often so as to be entirely surrounded by them, and with intertwining branches. When a form remains free of mildew under such favorable conditions for infection, it may be called immune, especially when the disease spreads as easily as in the case of powdery mildews.

A second objection to our experimental procedure has been based on the supposition that somewhere there might exist strains of *Erysiphe Polygoni* which would infect the so-called "immune" strains of Oenothera. This is quite possible but hardly concerns us, since we have not been interested particularly in the production of disease-free Oenothera strains, but rather in the fact that immunity to certain strains of Erysiphe exists, and that such immunity acts as a dominant unit factor in heredity. Our data in regard to the inheritance of immunity or susceptibility concern a *certain biologic strain of Erysiphe Polygoni*, abundant upon Oenothera at Ann Arbor, Michigan. Other strains of Erysiphe might conceivably infect our "immune" types. In general, however, the types immune at Washington, D. C., also proved to be immune in Michigan.

General Statements in Regard to the Crosses Made

Of the five strains of Oenothera selected, each was crossed with the four remaining ones, and each was self-pollinated; together there were five self-pollinations, and 20 cross-pollinations in the first season.

It might be asked if crosses between two immune or two susceptible strains would not be superfluous. The genetical relations of the Oenothera have been proved to be different in so many instances from those of other plants that all the possible crosses were made. The results obtained showed that in one case susceptible plants were obtained by crossing immune strains (in *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*). In no case were immune plants obtained in a cross between two susceptible species.

Before going into detail regarding the crosses made and the F_1 generations produced, it will be well to state that the system used of designating the crosses is the conventional one. The pistillate parent is always named first, followed by the name of the pollen parent.

Since the prevalence of zygotic sterility is surely significant in connection with the explanation of genetical phenomena in Oenothera, it is perhaps of interest to state the germination data for the seeds of the five strains. It should be strongly emphasized, however, that seeds of very low viability are usually those produced too late in the season to ripen normally. In other words, the high proportion of bad seeds is partly due to environmental factors. Abundant seeds were obtained in every case. The highest germination obtained was in *Oe. mississippiensis*, with 43 percent germination; the lowest was in one culture of *Oe. cinerescens*, with no germination, probably an example of the effect of immaturity; another culture of the same species, but from a different individual plant, showed about 10 percent germination. The other three strains germinated as follows: "*Oenothera biennis Chicago*," 23.6 percent; *Oe. pratincola* hyb. *immunis*, 29 percent; *Oe. pratincola* ("Lexington C"), 29.4 percent.

From each of the crosses, whenever possible, about 500 seeds were sown, and of the plants obtained 100 were potted off and later planted in the field. The data included in this paper extend to the F_2 generations obtained by the self-pollination of typical F_1 plants.

A few words in regard to metacliny will not be out of place at this time. As has been said, an Oenothera hybrid is an $\alpha\beta$ combination and usually similar in most of its characters to one of its parents. Sometimes in hybrid progenies a few plants are observed like the other reciprocal cross. These are metaclinic plants and are interpreted as $\beta\alpha$ combinations. In these cases the β gamete is the female (comes from the pistillate parent) and the α gamete is male (from the pollen parent).

Groups of Crosses

I. Crosses between *Oenothera mississippiensis* (susceptible) and *Oenothera cinerescens* (immune).

The F_1 plants produced in the cross *Oe. mississippiensis* × *Oe. cinerescens* were, in each of two crosses made, all of the *mississippiensis* type and showed abundant infection with mildew, except that in one of the progenies there was one metaclinic plant of the type of *Oe. cinerescens*, which was immune. The F_2 plants, obtained by self-pollination of typical F_1 plants from both crosses, were entirely similar to the F_1 plants, both in external morphological characteristics and in the degree of susceptibility.

In the reciprocal cross (*Oe. cinerescens* × *Oe. mississippiensis*) twin hybrids of the *Oe. cinerescens* type were obtained, both types immune to mildew. There was one metaclinic plant, of the type of *Oe. mississippiensis*, which was susceptible. The self-pollinated matroclinic plants of the F_1 gave no seeds, and the most essential data on the F_2 are therefore lacking. The single metaclinic plant, however, gave seeds by self-pollination and produced, in the F_2, susceptible plants similar to itself and to those of the cross *Oe. mississippiensis* × *Oe. cinerescens*.

II. Crosses between *Oenothera mississippiensis* (susceptible) and *Oenothera pratincola* hyb. *immunis* (immune).

In the cross *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*, all the plants of the F_1 produced were of the *mississippiensis* type, and immune towards powdery mildew. The F_2 plants were again of the *mississippiensis* type, with some slight morphological differences between two cultures coming from two different individuals of the same F_1 culture, which, however, were not detected as different when self-pollinated. All the plants obtained in both cultures were immune.

In the F_1 of the reciprocal cross (*Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*), all the plants with one exception were matroclinic, except for lack of mildew resistance. (The one exceptional plant was a mutation.) All the plants were susceptible and of the type of *Oe. pratincola*.

The F_2 plants from the reciprocal cross, *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*, were of three types, all closely resembling *Oe. pratincola*. All the plants were susceptible, as in the F_1.

III. Crosses between *Oenothera mississippiensis* (susceptible) and "*Oenothera biennis Chicago*" (susceptible).

In the cross *Oe. mississippiensis* × "*Oe. biennis Chicago*," all F_1 hybrids, with the exception of one, proved to be matroclinic, while the exceptional plant was metaclinic and of the *Oe. pratincola* type. (See description of "*Oe. biennis Chicago.*")

Among the F_1 hybrids of the reciprocal cross ("*Oe. biennis Chicago*" × *Oe. mississippiensis*), three plants of the *mississippiensis* type were produced, while the rest were of the "*Oe. biennis Chicago*" type, again showing matroclinic inheritance with a tendency toward metacliny. One mutation (of the *latifolia* type) was produced in one of these reciprocal crosses.

All the plants of this pair of reciprocals were mildewed. No difference seemed to exist in the degree of susceptibility, and, because both parents are susceptible in the same degree, no other data could be obtained on this point. The F_2 plants of both reciprocals were respectively of the same general type as the F_1, and all plants were susceptible.

IV. Crosses between *Oenothera mississippiensis* (susceptible) and *Oenothera pratincola* ("Lexington C") (susceptible).

The F_1 and F_2 generations of the cross *Oe. mississippiensis* × *Oe. pratincola* consisted of only one matroclinic type. All the plants were susceptible.

The F_1 of the reciprocal cross, *Oe. pratincola* × *Oe. mississippiensis*, was likewise of one general type, similar to *Oe. pratincola*, another illustration of matroclinic inheritance. A part of the plants, however (28 out of 100), showed a distinct yellowish-green coloring and mottling of the leaves, in some cases going over to white, especially at the margins of the leaves. On this account the culture might be interpreted as consisting of very closely similar twin hybrids, both, however, resembling the pistillate parent (*Oe. pratincola*) in external characters, and probably only slightly different in genetical constitution. All the plants were susceptible, somewhat more so than those of the reciprocal. The F_2 repeated the two types of the F_1, with some slight segregation in morphological characters, but all plants were susceptible.

V. Crosses between "*Oenothera biennis Chicago*" (susceptible) and *Oenothera cinerescens* (immune).

All the plants of the F_1 produced from the cross "*Oe. biennis Chicago*" × *Oe. cinerescens* were of the *Oe. pratincola* type. (See description of "*Oe. biennis Chicago.*") All were susceptible. No seeds were obtained by self-pollination, and consequently no F_2 can be reported.

The F_1 plants of the reciprocal (*Oe. cinerescens* × "*Oe. biennis Chicago*") were all of the *cinerescens* type, with the exception of two metaclinic plants of the *pratincola* type. All *cinerescens*-like plants were immune, in both the F_1 and the F_2. Both metaclinic plants were resistant.

VI. Crosses between "*Oenothera biennis Chicago*" (susceptible) and *Oenothera pratincola* hyb. *immunis* (immune).

The cross "*Oe. biennis Chicago*" × *Oe. pratincola* hyb. *immunis* gave an F_1 generation of one type (similar to *Oe. pratincola*). All the plants were immune to mildew. No F_2 was obtained.

The F_1 plants of the reciprocal cross (*Oe. pratincola* hyb. *immunis* × "*Oe. biennis Chicago*") were likewise of one type (*Oe. pratincola*). All the plants obtained were susceptible to mildew, and gave an identical, susceptible F_2.

In these crosses the factors determining immunity are in the β gametes, and immunity is therefore a patroclinic character.

VII. Crosses between "*Oenothera biennis Chicago*" (susceptible) and *Oenothera pratincola* ("Lexington C") (susceptible). These crosses offer the same difficulty as the former in regard to the differentiation of types in the progenies, since the parents are themselves doubtfully distinguishable.

In the cross "*Oe. biennis Chicago*" × "Lexington C," all F_1 plants with the exception of three were *pratincola*-like, the three exceptions being mutations. Two of these were similar (probably of one type) and showed a slight susceptibility towards mildew. The third was of a different type and was very susceptible towards mildew. All the typical plants were highly susceptible. The F_2 was the same as the F_1. In the reciprocal cross ("Lexington C" × "*Oe. biennis Chicago*") all plants of the F_1 and F_2 generations were of one type and very susceptible.

The only statement that can be made in regard to inheritance of susceptibility in these crosses in which both parents are highly susceptible is that the offspring are likewise highly susceptible. The two slightly susceptible mutations show that a marked degree of resistance may be acquired as a result of mutational change.

VIII. Crosses between *Oenothera cinerescens* (immune) and *Oenothera pratincola* hyb. *immunis* (immune).

The F_1 generation of the cross *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* consisted of two *cinerescens* types, one of them being similar to *Oe. cinerescens* in nearly all respects, the other a smaller or dwarf type. All plants were immune, and gave an immune F_2 like the F_1, with a few mutations.

In the reciprocal cross (*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*) the F_1 generation consisted of only one type of plants (a small *pratincola* type), all of which were susceptible to mildew. The F_2 showed a splitting into two types, one more delicate than the other, but both *pratincola*-like and both heavily mildewed.

These crosses show conclusively that the female α gametes of the hyb. *immunis* do not carry factors for immunity, but that the male β gametes do. Consequently, any combination to which hyb. *immunis* contributes the egg will be immune only providing the pollen parent produces male gametes with the immunity factor.

IX. Crosses between *Oenothera cinerescens* (immune) and *Oenothera pratincola* ("Lexington C") (susceptible).

All the F_1 plants produced from the cross *Oe. cinerescens* × "Lexington C" were of the *Oe. cinerescens* type and similar to the larger one of the twin hybrids produced in the cross *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*.

All plants produced were immune to mildew. The F_2 plants were similar to the F_1 and immune.

In the reciprocal cross, "Lexington C" × *Oe. cinerescens*, all F_1 plants were of the *Oe. pratincola* type and susceptible. The F_2 split into two types, both in general like *Oe. pratincola*. All the plants of both types were susceptible.

X. Crosses between *Oenothera pratincola* hyb. *immunis* (immune) and *Oenothera pratincola* ("Lexington C") (susceptible).

All F_1 and F_2 plants of the cross *Oe. pratincola* hyb. *immunis* × "Lexington C" were of the type of *Oe. pratincola* and susceptible, while all plants in the F_1 of the reciprocal cross, *Oe. pratincola* × *Oe. pratincola* hyb. *immunis*, were of the hyb. *immunis* type and immune. Among the F_2 plants of the reciprocal were several mutations and a few metaclinic plants, the latter being highly resistant. All other plants, including the mutations, were immune.

Conclusions

In several of the foregoing cases in which metaclinic plants were produced in crosses between immune and susceptible parents, the immunity-factor combination which would insure susceptibility or immunity in one particular type seems not to insure the same effect in another type. Investigations are now started to prove, in these cases, whether or not the expression of the immunity factors is influenced by morphological characters. In other words, is it possible that types may exist in which susceptibility is so great that one **I** factor will not confer immunity, whereas in other types the factor complex, without **I**, is so highly resistant that the addition of **I** confers complete immunity? The explanation of the phenomena presented by metaclinic plants must be deferred. As far as the normal hybrids are concerned, the results are all consistent and lead to definite conclusions.

The results accord perfectly with the following hypotheses:

1. The factor for immunity (**I**) is dominant. If it enters the zygote from either side, the plant produced is immune.

2. In all the five strains involved in the experiments, the eggs are different from the sperms. The former are α gametes, the latter β gametes. A few exceptions to this general rule are indicated by the rare appearance of metaclinic plants in the progenies. Whereas a normal hybrid is an $\alpha\beta$ combination, the metaclinic hybrid is $\beta\alpha$.

3. In both the immune strains, the immunity is due to an unbalanced factor for immunity in the zygote. In *Oe. cinerescens* this factor is strictly associated with the α gamete, and in *Oe. pratincola* hyb. *immunis* with the β gamete.

4. Representing immunity and susceptibility by capital **I** and small **i** respectively, the zygotic composition and reaction to mildew of the five strains are as follows:

Oe. pratincola hyb. *immunis*, αiβI, immune.
Oe. cinerescens, αIβi, immune.
Oe. mississippiensis, αiβi, susceptible.
"*Oe. biennis Chicago*" (a strain of *Oe. pratincola*), αiβi, susceptible.
Oe. pratincola ("Lexington C"), αiβi, susceptible.

5. The composition and reaction to mildew of the several F_1 hybrids must therefore be as formulated below:

Oe. mississippiensis × *cinerescens*, αiβi, susceptible.
Oe. cinerescens × *mississippiensis*, αIβi, immune.
Oe. mississippiensis × *Oe. pratincola* hyb. *immunis*, αiβI, immune.
Oe. pratincola hyb. *immunis* × *Oe. mississippiensis*, αiβi, susceptible.
Oe. mississippiensis × "*Oe. biennis Chicago*," αiβi, susceptible.
"*Oe. biennis Chicago*" × *Oe. mississippiensis*, αiβi, susceptible.
Oe. mississippiensis × *pratincola*, αiβi, susceptible.
Oe. pratincola × *Oe. mississippiensis*, αiβi, susceptible.
"*Oe. biennis Chicago*" × *cinerescens*, αiβi, susceptible.
Oe. cinerescens × "*Oe. biennis Chicago*," αIβi, immune.
"*Oe. biennis Chicago*" × hyb. *immunis*, αiβI, immune.
Oe. pratincola hyb. *immunis* × "*Oe. biennis Chicago*," αiβi, susceptible.
"*Oe. biennis Chicago*" × *pratincola*, αiβi, susceptible.
Oe. pratincola × "*Oe. biennis Chicago*," αiβi, susceptible.
Oe. cinerescens × hyb. *immunis*, αIβI, immune.
Oe. pratincola hyb. *immunis* × *Oe. cinerescens*, αiβi, susceptible.
Oe. cinerescens × *Oe. pratincola*, αIβi, immune.
Oe. pratincola × *Oe. cinerescens*, αiβi, susceptible.
Oe. pratincola hyb. *immunis* × *pratincola*, αiβi, susceptible.
Oe. pratincola × hyb. *immunis*, αiβI, immune.

In every case the reaction of the hybrid to mildew conformed exactly to expectations, according to the formulation above.

6. On account of their peculiar type of heterogametism, immunity due to a single factor must breed as true as that due to a factor pair. Of the total number of 20 hybrids, 13 were susceptible, 3 had a single factor for immunity, derived from the maternal parent, 3 had a single factor for immunity, derived from the paternal parent, and one only had double immunity, derived from both parents.

7. In accord with the hypothesis of immunity advanced above, combined with the hypothesis of heterogametism, the F_2 generation by self-pollination of F_1 plants should be the same, in regard to immunity or susceptibility, as the F_1. This conclusion has been amply proved.

LITERATURE CITED

1. **Atkinson, G. F.** Sorting and blending of unit characters in the zygote of Oenothera with twin and triplet hybrids in the first generation. Zeitschr. Indukt. Abstamm.- u. Vererbungsl. **16**: 193–238. 1916.
2. ——. Quadruple hybrids in the F_1 generation from *Oenothera nutans* and *Oenothera pycnocarpa* with the F_2 generations and back and inter-crosses. Genetics **2**: 213–260. 1917.
3. **Bartlett, H. H.** The status of the mutation theory, with especial reference to Oenothera. Amer. Nat. **50**: 513–529. 1916.

Smithsonian Institution
OCT 20 1922
National Museum

4. ——. Twelve elementary species of Onagra. Cybele Columbiana **1**: 37–56. 1914.
5. ——. Systematic studies on Oenothera VI. *Oe. cinerescens* and *Oe. mississippiensis*, spp. novv. Rhodora 24: ——. 1922. (In press.)
6. **Cobb, Frieda, and Bartlett, H. H.** On Mendelian inheritance in crosses between mass-mutating and non-mass-mutating strains of *Oenothera pratincola.* Jour. Wash. Acad. Sci. **9**: 462–483. 1919.
7. **Salmon, E. S.** A monograph of the Erysiphaceae. Mem. Torr. Bot. Club **9**. 1900.
8. ——. Cultural experiments with the barley-mildew, *Erysiphe graminis* DC. Ann. Mycol. **2**: 70–99. 1904.
9. ——. Cultural experiments with an Oidium on *Euonymus japonicus* Linn. Ann. Mycol. **3**: 1–15. 1905.
10. ——. Cultural experiments with "biologic forms" of the Erysiphaceae. Phil. Trans. Roy. Soc. London B, **197**: 107–122. 1905.
11. **Searle, G. O.** Some observations on *Erysiphe Polygoni* DC. Trans. Brit. Mycol. Soc. **6**: 274–293. 1920.
12. **de Vries, H.** *Oenothera nanella,* healthy and diseased. Science n. ser. **35**: 753–754. 1912.
13. ——. Gruppenweise Artbildung unter spezieller Berücksichtigung der Gattung Oenothera. Berlin, 1913.

GROWTH OF SOME PARASITIC FUNGI IN SYNTHETIC CULTURE MEDIA

H. C. Young and C. W. Bennett [1]

(Received for publication January 7, 1922)

In some studies on parasitism being conducted by the writers, it early became imperative that considerable attention be given to synthetic nutrient solutions for fungi. Evidence points strongly to food as being at least one of the dominant factors in the type of growth produced, and in the variations obtained. The presence or absence of any of the essential inorganic elements may vary the growth forms to such an extent that structural changes may develop. Striking responses to chemicals have been noted, in color changes as shown by Milburn (8) and Bessey (1), by generic changes as in the presence or absence of setae in *Colletotrichum lindemuthianum*, and of conidial chains in *Alternaria brassicae* and *Macrosporium brassicae*, as reported by Stevens (15), and by changes in the hydrogen-ion concentration during growth of the organism. The last is undoubtedly a factor in parasitism.

The work of Pasteur (10) on yeasts led to a very extensive investigation of the food requirements of fungi. The early investigators dealt in a minor way with the inorganic foods, more emphasis being given to the organic compounds which could be used as a source of carbon. It was early recognized, however, that for a detailed study of any fungus the food supply had to be fairly definitely controlled and that synthetic solutions rather than plant decoctions should be used.

Of the commonly used synthetic solutions, that developed by Raulin (12)[2] is the earliest. The solution contains most of the elements found in the ash of fungi. For the most part it was developed to meet the requirements for the growth of *Aspergillus niger* and of some other strict saprophytes.

---

[1] Contribution from the Department of Botany, Michigan Agricultural College.

[2] Raulin's synthetic solution:

Ammonium nitrate	4.0 g.
Ammonium phosphate	0.6 g.
Ammonium sulphate	0.25 g.
Potassium silicate	0.07 g.
Potassium carbonate	0.4 g.
Zinc sulphate	0.07 g.
Ferric sulphate	0.07 g.
Magnesium carbonate	0.4 g.
Saccharose	70.00 g.
Tartaric acid	4.00 g.
Water	1500.00 cc.

Mayer (7)[3] prepared a similar solution, eliminating zinc, iron, and silicon, but including calcium acid phosphate in addition to the other elements.

Simpler synthetic solutions were later devised by Pfeffer (11),[4] Richards (13),[5] Currie (3),[6] Uschinsky (5),[7] and Czapek (4),[8] and these are frequently used in present-day studies.

[3] Mayer's synthetic solution:

Magnesium sulphate	2.5 g.
Ammonium nitrate	10.0 g.
Calcium phosphate, $Ca_3(PO_4)_2$	2.5 g.
Potassium acid phosphate (monobasic)	5.0 g.
Cane sugar	50.0 g.
Water	1000.0 cc.

[4] Pfeffer's solution:

Ammonium nitrate	10.0 g.
Potassium phosphate (monobasic)	5.0 g.
Magnesium sulphate	2.5 g.
Cane sugar	50.0 g.
Ferrous sulphate	trace
Water	1000. cc.

Reaction, pH = 4.3

[5] Richards' solution:

Potassium nitrate	1. g.
Potassium acid phosphate (monobasic)	0.5 g.
Magnesium sulphate	0.25 g.
Ferric chloride	trace
Saccharose	3.43 g.
Water	100. cc.

Reaction, pH = 4.2

[6] Currie's solution:

Ammonium nitrate	2. g.	2.5 g.
Potassium acid phosphate (monobasic)	.75 g.	1.0 g.
Magnesium sulphate	.25 g.	.25 g.
Cane sugar	125.00 g.	150.00 g.
Water	1000 cc.	

Reaction (add 4 cc. of $N/5$ HCl), pH = 3.4–3.6

[7] Uschinsky's solution:

Ammonium lactate	6.7 g.
Sodium asparaginate	3–4. g.
Potassium acid phosphate (monobasic)	2–2.5 g.
Magnesium sulphate	0.3–0.4 g.
Sodium chloride	5–7. g.
Calcium chloride	0.1 g.
Glycerin	30–40. g.
Water	1000. cc.

[8] Czapek's solution:

Magnesium sulphate	0.5 g.
Potassium acid phosphate (monobasic)	1.0 g.
Potassium chloride	0.5 g.
Sodium nitrate	2.0 g.
Cane sugar	3.4 g.
Water	100. cc.

Reaction, pH = 6.8

Coons (2),[9] working with *Plenodomus fuscomaculans*, recognized that a medium favorable to vegetative growth is not necessarily conducive to sporulation. He found it necessary to develop two solutions, one inducing mycelial development and the other favoring spore production.

In a general way all the above mentioned solutions are similar. Emphasis is most frequently placed on the source of carbon. In no case is there given a method for the arranging of the proportions and concentrations. The presence or absence of any one of the inorganic constituents depends upon whether the investigator considered it important for the growth of the particular fungus under experimentation. Most discussion, however, has centered around the rôles of calcium, iron, and zinc.

Molisch (9) is of the opinion that calcium is not necessary for the normal development of fungi. While this fact is based on little experimental data, it has been generally accepted.

Currie (3) has concluded that the exclusion of iron is without effect on the growth of *Aspergillus niger*. Other investigators have found that, although iron and calcium may not be elements essential for growth, they unquestionably exert a beneficial effect on the growth of many fungi. More recently the work of Steinberg (14) has shown that in the case of *Aspergillus niger*, zinc has a very decided stimulative effect on the quantity of fungus produced. He states that one reason that zinc has not previously been used in such media is that sufficient amounts were dissolved from the glassware to supply this need. This same statement might well be made in the case of iron and possibly in that of some of the other essential inorganic elements.

It is worthy of note that nearly all the synthetic solutions now in general use have been formulated on the basis of the food requirements of strict saprophytes, in most cases with *Aspergillus niger*, a fungus that is not at all selective but will grow on an exceptionally wide range of media. Moreover, the name *Aspergillus niger* as used by different investigators represents rather a group of related fungi than one species. For these reasons, a study of the rôle of any salt cannot be safely made when such types of fungi are used as criteria.

[9] Coons' cheap synthetic solution:

Magnesium sulphate	1.23 g.
Potassium acid phosphate (monobasic)	2.72 g.
Potassium nitrate	2.02 g.
Maltose	7.2 g.
Water	1000. cc.

Coons' synthetic solution:

Magnesium sulphate	1 cc. of $M/5$ solution
Potassium acid phosphate	5 cc. of $M/5$ solution
Asparagin	1 cc. of $M/5$ solution
Maltose	5 cc. of $M/5$ solution
Water	50 cc.

The growing of strictly parasitic fungi in synthetic culture solutions has not come into general practice. On the contrary, various circumstances have led to the general use of plant decoctions. Such media are excellent for certain purposes, but for physiological work media of known composition are almost indispensable.

In an attempt to formulate a synthetic solution for the growth of parasitic fungi, the rôles of the so-called essential inorganic elements along with that of calcium and zinc were studied. For this work Richards' solution was used as a standard and for the deviations. The inorganic constituents were replaced by non-essentials, the concentrations and balance being maintained as nearly as possible. In addition, eight sugars were used. The possible importance of the presence of minute quantities of zinc and of other substances was recognized, and special precautions were taken in selecting glass of tested solubility and in its thorough cleaning. Erlenmeyer flasks of 150-cc. capacity were employed. The water used was stock distilled water redistilled through a Branstead still. The chemicals were obtained from the J. T. Baker Chemical Company and from the lot prepared for the use of the National Research Council in nutrition studies of higher plants. The arrangement of the experiment is given in table 1. In each flask was placed 50 cc. of the nutrient solution, and each solution was set up in three sets of triplicates. The solutions were sterilized by heating in an autoclave 30 minutes at 10 pounds' pressure. One series of flasks was inoculated with *Fusarium oxysporum*, another with *Rhizopus nigricans*, and a third with *Aspergillus niger*. After the flasks were inoculated, they were placed at room temperature and the organisms were allowed to grow for 16 days. At the end of this period the mycelium and spores were collected on previously dried and weighed filter paper, dried to constant weight in a steam oven at 97° C., and reweighed. Results are recorded in table 1.

The results of this experiment indicate that calcium exerts a stimulative action on the growth of the three organisms used. The exact manner in which calcium influences growth is problematical. However, it can be logically concluded that its rôle of counteracting acidity in higher plants plays an important part in the growth of these fungi, especially in that of *Aspergillus niger* and *Rhizopus nigricans*, two acid producers. To make certain that the increased weight was due to fungous growth and not to calcium oxalate crystals, the mycelium was treated with alcohol and thirty percent hydrochloric acid and redried. The treatment resulted in no appreciable decrease in weight of the fungous material grown with calcium present, as compared with the cultures grown without calcium. The quantity of acid produced by the growth of *Aspergillus niger* and *Rhizopus nigricans* is proportional to the amount of fungous material produced. With available carbon, growth continues until stopped by the hydrogen-ion concentration. If the acid is neutralized, *Rhizopus nigricans* will grow until

TABLE I

No. of Solution	Composition of Culture Solution	*Fusarium oxysporum*, Dry Weight in Grams*	pH	*Aspergillus niger*, Dry Weight in Grams*	pH	*Rhizopus nigricans*, Dry Weight in Grams*	pH
1	Richards' solution	.2094	5.8	.5450	2.4	.2015	2.6
2	Richards' solution, KNO_3 replaced by $Ca(NO_3)_2$	.2494	6.4	.8070	4.8	.2747	
3	Richards' solution, KH_2PO_4 replaced by Na_2HPO_4	.2575	6.4	.3841	2.6	.1843	
4	Richards' solution, KH_2PO_4 replaced by KCl	.0736	4.4	.1641		.0925	
5	Richards' solution, KNO_3 replaced by KCl	No growth		.0250	4.6	No growth	
6	Richards' solution, sucrose replaced by dextrose	.1932	5.6	.3700	3.6	.1234	
7	Richards' solution, sucrose replaced by maltose	.3124	6.4	.3560	2.4	.1540	
8	Richards' solution, sucrose replaced by lactose	.0404	5.0	.2640	4.0		
9	Richards' solution, sucrose replaced by glycerin	.1163	4.2	.0406			
10	Richards' solution, sucrose replaced by raffinose			.2900	2.4	.1000	
11	Richards' solution, sucrose replaced by galactose	.2345	6.0	.4240	2.6	.1560	
12	Richards' solution, sucrose replaced by levulose	.1132	4.2	.1690	3.0	.0825	
13	Richards' solution, sucrose replaced by mannose	.2146	5.8	.4040	3.2	.1420	
14	Richards' solution, iron omitted	.2458	6.4	.7243	3.6		
15	Richards' solution, plus 15 mg. $ZnSO_4$	.2432	6.4	.7131			
16	" " " 10 " "	.2243	6.2	.9043	2.4		
17	" " " 5 " "	.2186	6.3	.8078	2.4		
18	" " " 1 " "	.2506	6.6	.8124	2.4		
19	" " " 0.5 " "			.6312	2.8		
20	Coons' cheap synthetic solution	.0500	3.8				

* Average for three cultures.

the available carbon is completely used up. Our results in this connection are contrary to those of Graves (6), who states that a thermolabile "staling" substance is produced which stops the growth of the fungus.

The reaction of the culture solution in which *Fusarium oxysporum* was grown continued to become acid until a hydrogen-ion concentration of pH 3.6 was reached, then turned alkalineward, and growth continued until all the organic compounds were broken up and a hydrogen-ion concentration of pH 8.4 was reached.

Zinc sulphate stimulated growth in the case of *Aspergillus niger* but not in that of *Fusarium oxysporum* or of *Rhizopus nigricans*.

Since calcium proved to be so beneficial when placed in the nutrient solution for the growth of the organisms used in the preceding experiment, it was thought important to determine whether this same relation would hold for a number of parasitic organisms. An experiment was planned using Richards' solution as a medium in one series and the same solution except with calcium nitrate replaced by potassium nitrate in a second series. A third series with zinc sulphate added to Richards' solution was arranged. These series were prepared in triplicate and with the same precautions as used in the preceding experiment. The organisms used were selected to represent as wide a range of parasites as could be obtained from the stock cultures of the laboratory. The experiment was continued for each organism until growth seemed to stop. The organisms used and the results are tabulated in table 2.

TABLE 2

Organism	Richards' Solution, Calcium Nitrate Substituted for Potassium Nitrate		Richards' Solution		Richards' Solution Plus 5 Mg. Zinc Sulphate	
	Dry Wt.*	pH	Dry Wt.*	pH	Dry Wt.*	pH
Botrytis allii	.2199	4.2	.2404	5.8	.2191	6.3
Phoma apiicola	.2798	5.2	.1427	6.0	.1963	6.0
Fusarium conglutinans	.1628	6.0	.1669	6.4	.1560	6.6
Cercospora apii	.3750	5.2	.3003	5.4	.3509	5.4
C. beticola	.3451	6.4	.3411	6.8	.3118	6.8
Fusarium batatatis	.2391	6.1	.2377	6.3	.2501	8.0
F. radicicola	.2666	8.0	.3224	6.8	.2561	7.0
Sclerotinia libertiana (strain 1)	.4545	3.8	.4675	4.2	.4391	3.8
S. cinerea	.2132					
S. libertiana (strain 2)	.1963	3.0	.2153	3.0	.2052	3.0
Rhizoctonia solani	.1055		No growth		No growth	
Macrosporium sarcinaeforme	.2777	6.8	.2801	6.6	.2683	6.6
Sphaeropsis malorum	.3139	3.6	.3751	6.8	.3689	6.8
Sterigmatocystis violae	.3264	6.0	.2884	6.0	.2435	6.0
Vermicularia sp.	.3272		.3563		.3363	
Ascochyta pisi	.2677	6.2	.3782	6.4	.3278	8.0
Colletotricum lagenaria	lost					
Dothidella quercus	.3652	6.6	.2411	6.5	.2999	6.5

* Average for three cultures.

Summarizing the results of table 2, calcium is found to be generally beneficial. In the case of four organisms it retarded growth. In the remaining cultures it was stimulative or exerted no action. *Rhizoctonia solani* grew only when calcium was present. The importance of the rôle of calcium, however, cannot be estimated from the results of this experiment, as we have no proof that we have been using it in its proper proportions.

Zinc sulphate gave slightly beneficial effects with only two organisms, *Dothidella quercus* and *Phoma apiicola.*

A striking example of color formation was shown in the case of *Fusarium radicicola* which was pink in the solution containing calcium and colorless in potassium solutions. The color was destroyed when hydrochloric acid was added and could not be obtained again by the addition of sodium hydroxide.

The results of the above described experiment indicate that calcium might be classed as one of the essential inorganic elements in synthetic solutions for some fungi. Moreover, it would seem that the salt requirements for an optimum synthetic solution would be composed of the six elements, nitrogen, phosphorus, sulphur, potassium, calcium, and magnesium.

We have no evidence to the effect that we have been using these inorganic elements in correct proportions. Since the salt requirements for fungi are probably the same as for higher plants, a series of culture solutions was arranged according to the method used by the National Research Council for work in the study of nutrition in higher plants. A triplicate series of twenty-one cultures, using potassium acid phosphate (monobasic), calcium nitrate, and magnesium sulphate, was arranged. The salts varied in the different solutions by increments of one eighth and had an osmotic concentration of three and one half atmospheres. Sucrose was added in equal amounts of 3.43 grams per 100 cc. of the solution, thereby giving the culture solutions a total osmotic concentration of four and one half atmospheres. Similarly, a triplicate series was arranged, using potassium nitrate, calcium acid phosphate, and magnesium sulphate.

TABLE 3

No.	Cc. of $M/14$ KH_2PO_4	Cc. of $M/14$ $Ca(NO_3)_2$	Cc. of $M/14$ $MgSO_4$	Cc. of Sugar Sol., 3.43 g. in 10 cc.	Water to make 50 cc.	Cc. of $M/3.5$ KNO_3	Cc. of $M/35$ $Ca(H_2PO_4)_2$	Cc. of $M/3.5$ $MgSO_4$	Cc. of Sugar Sol., 3.4 g. in 10 cc.	Water to make 50 cc.
11	4	4	22.4	5	14.6	.95	9.5	5.7	5	28.85
12	3.5	6.8	17.2	5	17.5	.91	18.6	4.6	5	20.89
13	3.4	10	13.2	5	18.4	.84	25.6	3.43	5	15.13
14	3.1	12.4	9.4	5	20.1	.81	32.6	2.45	5	9.14
15	3.1	15	6	5	20.9	.74	37.11	1.47	5	5.68
16	3.0	17	2.8	5	22.2	.73	43.75	.74	5	.00
21	7.4	4	17.2	5	16.4	1.89	9.5	4.62	5	29.03
22	7.0	6.8	13.2	5	18.0	1.68	18.6	3.43	5	21.39
23	6.5	10.0	9.4	5	19.6	1.58	25.6	2.45	5	17.27
24	6.2	12.4	6.0	5	20.4	1.47	32.6	1.47	5	9.46
25	6.0	15.0	2.8	5	21.2	1.44	37.1	.74	5	5.72
31	10.6	4.0	13.2	5	17.2	2.63	9.5	3.43	5	29.44
32	10.0	6.8	9.4	5	18.8	2.45	18.6	2.45	5	21.50
33	9.5	10.0	6.0	5	19.5	2.35	25.6	1.47	5	15.58
34	9.0	12.4	2.8	5	20.8	2.24	32.6	.74	5	9.42
41	13.8	4.0	9.4	5	17.8	3.47	9.5	2.45	5	29.58
42	13.2	6.8	6.0	5	19.0	3.25	18.6	1.47	5	21.68
43	12.6	10.0	2.8	5	19.6	2.98	25.6	.74	5	15.68
51	17.2	4.0	6.0	5	17.8	4.38	9.5	1.74	5	29.65
52	16.4	6.8	2.8	5	19.0	3.96	18.6	.74	5	21.70
61	20.3	4.0	2.8	5	17.9	4.87	9.5	.74	5	29.89

Osmotic concentration, 4.5 atm.; pH, 4.8–5.2.

The method of arranging the concentrations is given in table 3. The inorganic salts used were of the highest purity of the grade mentioned above. The technique used was the same as in the preceding experiments. Special care was taken in inoculation of solutions so as to obtain uniform growths at the outset. *Fusarium oxysporum*, *Macrosporium sarcinaeforme*, and *Phoma apiicola* were used. Richards', Pfeffer's, Currie's, Meyer's, and Czapek's solutions were used as checks.

Dry weights were obtained after the cultures had grown for sixteen days. The hydrogen-ion concentration of each culture solution was determined at the time the experiment was concluded. The results obtained are given in table 4.

TABLE 4

KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$, KNO_3, $Ca(H_2PO_4)_2$, $MgSO_4$

No. of Culture	*Fusarium oxysporum*			*Fusarium oxysporum*			*Macrosporium sarcinaeforme*			*Phoma apiicola*		
	Dry Wt.*	pH	Rank	Dry Wt.*	pH	Rank	Dry Wt.*	pH	Rank	Dry Wt.*	pH	Rank
11	.2159	5	11	.1514	3.6	15	.4311	6	17	.3448	6.3	7
12	.2163	6.3	10	.1422	3.6	17	.3862	5.7	20	.1627	5.8	12
13	.2221	6.4	9	.1369	3.8	18	.4565	5.4	16	.1514	4.8	14
14	.1876	3.6	17	.1216	4.0	19	.4085	6.2	18	.1567	3.8	13
15	.1765	5.4	19	.1160	4.0	20	.3414	5.0	21	.1019	3.5	19
16	.1895	6.4	16	.0985	4.6	21	.3902	5.0	19	.0269	3.4	21
21	.2029	3.9	13	.2133	5.4	21	.6888	7.0	11	.5301	6.5	3
22	.1755	6.2	20	.2326	5.8	9	.8002	6.0	8	.1265	4.4	16
23	.2246	6.4	7	.2089	5.0	12	.6708	5.2	13	.1764	3.8	11
24	.2601	5.4	2	.1898	5	14	.4904	4.8	15	.0815	3.8	20
25	.2548	4.6	3	.1457	5	15	.6352	5	14	.1268	3.6	15
31	.1835	5.9	18	.2137	6.4	10	.7685	7.4	9	.4412	6.5	6
32	.2761	6	1	.2509	6.2	7	.8195	6.8	6	.6531	6.2	1
33	.2412	6	4	.2463	5.4	8	1.0415	5.4	3	.3128	4.6	8
34	.2334	4.8	5	.1939	5	13	.900	5.0	5	.1141	4.6	18
41	.1962	5.9	15	.2718	6.4	5	.6849	7.2	12	.5864	6.5	2
42	.2317	5.6	6	.2588	6.0	6	1.0149	7.0	4	.5081	6.8	4
43	.4988	5.0	14	.2752	5.4	3	1.2294	6.2	1	.1161	4.6	17
51	.2236	5.8	8	.2727	6.5	4	.7403	7.2	10	.4805	6.2	5
52	.2061	5.6	12	.3183	6.7	1	1.0735	7.2	2	.3031	6.2	9
61	.1399	5.2	21	.2885	6.8	2	.8054	8.4	7	.2531	6.2	10
Richards	.1995	4.8					.4515	6.8		.0952	5.6	
Pfeffer							.0973	4.6		.0497	3.8	
Currie							.0144	3.2				
Meyer							.4187	5.2		.1473	3.8	
Czapek							.1053	7.4		.3126	6.8	

* Average of three cultures.

Figures 1, 2, 3, and 4 represent the arrangement of the triangular system. The area within the dotted line in each case represents the seven cultures making the best growth.

The results of this experiment show clearly that a proper balance of the inorganic constituents in the solution is very essential, and that this balance can be readily obtained by the use of the triangular system. Using approximately the same concentration as Richards' solution and a constant source of carbon, the growth obtained in the balanced solutions was much superior for every organism used in the experiment.

SUMMARY AND CONCLUSIONS

1. Various synthetic solutions have been evolved to meet the needs of specific organisms. Fungi vary so much in their mineral and food re-

quirements that no one medium can be made to give satisfactory results with all organisms.

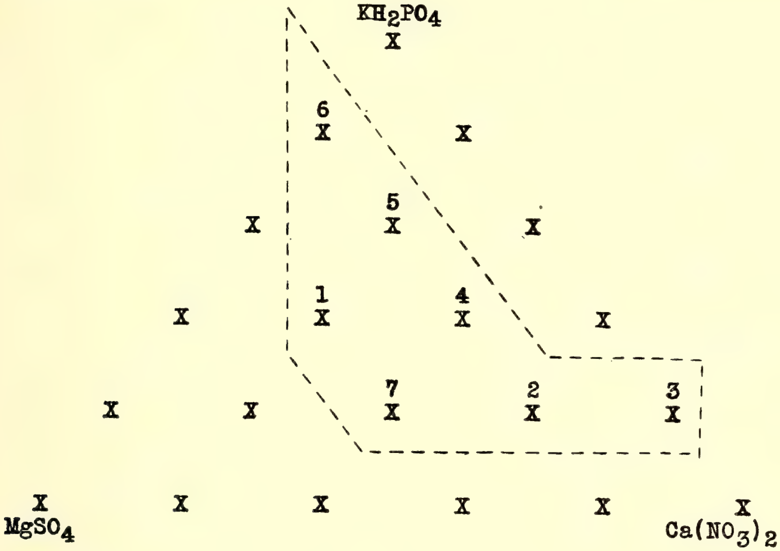

KH_2PO_4
6
5
1
4
7
2
3
$MgSO_4$
$Ca(NO_3)_2$

Fig. 1.
Fusarium oxysporum.

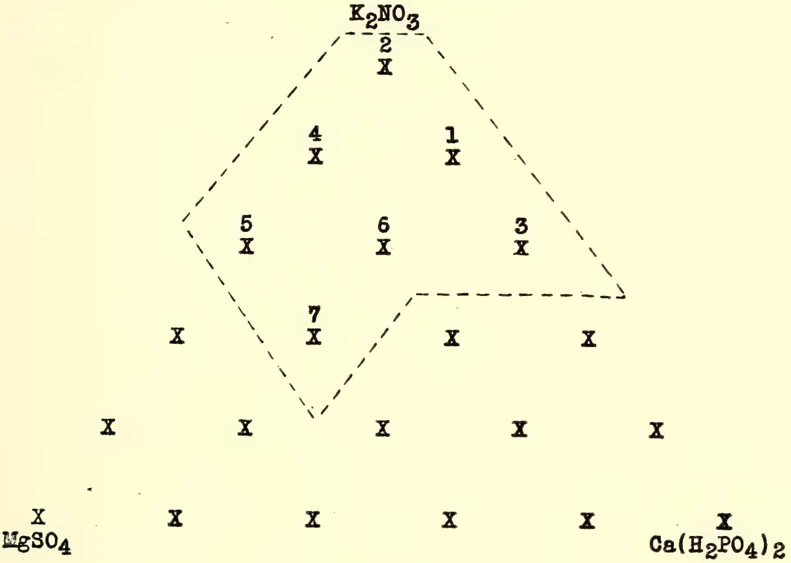

K_2NO_3
2
4
1
5
6
3
7
$MgSO_4$
$Ca(H_2PO_4)_2$

Fig. 2.
Fusarium oxysporum.

2. Zinc has a stimulating effect in the case of a limited number of organisms. In the case of some 15 species of parasitic organisms, zinc did not accelerate growth.

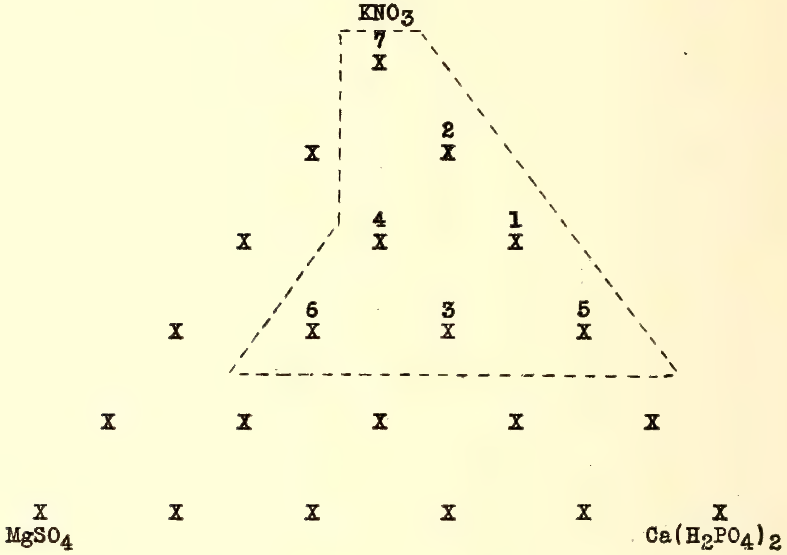

KNO_3
7
2
4
1
6
3
5
$MgSO_4$
$Ca(H_2PO_4)_2$

Fig. 3.
Macrosporium sarcinaeforme.

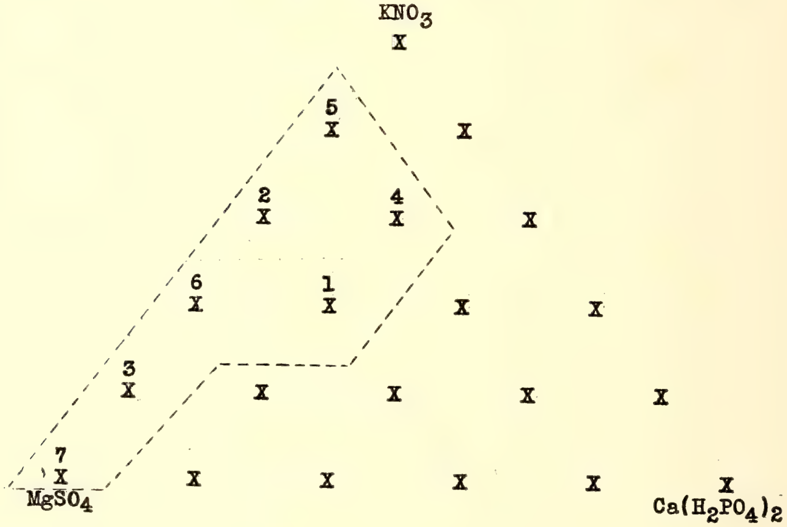

KNO_3
5
2
4
6
1
3
7
$MgSO_4$
$Ca(H_2PO_4)_2$

Fig. 4.
Phoma apiicola.

3. Calcium, in the case of the organisms used, proved to be beneficial in the majority of instances and seems to have a place, probably in the correcting of acidity, in the best synthetic solutions.

4. The inorganic elements in a synthetic solution should be properly balanced. The balancing of these salts is a comparatively simple procedure when the three-salt or triangular system is used. By this system the mineral requirements of any particular fungus can be quickly and accurately ascertained. By then selecting the sugar most readily used, a favorable culture solution can be made.

BIBLIOGRAPHY

1. **Bessey, E. A.** Ueber die Bedingungen der Farbbildung bei Fusarium. Flora **93**: 301–344. 1904.
2. **Coons, G. H.** Factors involved in the growth and pycnidium production of *Plenodomus fuscomaculans*. Jour. Agr. Res. **5**: 713–769. 1916.
3. **Currie, J. N.** The citric acid fermentation of *Aspergillus niger*. Jour. Biol. Chem. **31**: 15. 1917.
4. **Dox, A. W.** The intracellular enzymes of Penicillium and Aspergillus. U. S. Dept. Agr. Bur. Anim. Ind. Bull. **120**: 1–70. 1910.
5. **Duggar, B. M.** Fungous Diseases of Plants, p. 26. 1909.
6. **Graves, A. H.** Chemotropism in *Rhizopus nigricans*. Bot. Gaz. **52**: 337–370. 1916.
7. **Mayer, A.** Untersuchungen über die alkoholische Gährung, den Stoffbedarf und den Stoffwechsel der Hefepflanze. Heidelberg, 1879.
8. **Milburn, T.** Ueber Aenderungen der Farben bei Pilzen und Bakterien. Centralbl. Bakt. II, **13**: 258–276. 1904.
9. **Molisch, H.** Die mineralische Nahrung der niederen Pilze. Bot. Centralbl. **60**: 167, 168. 1894.
10. **Pasteur, L.** Mémoire sur la fermentation alcoölique. Ann. Chem. Phys. III, **58**: 323. 1860.
11. **Pfeffer, W.** Ueber Election organischer Nährstoffe. Jahrb. Wiss. Bot. **28**: 205–268. 1895.
12. **Raulin, J.** Études chimiques sur la végétation. Ann. Sci. Nat. Bot. V, **11**: 93–299. 1869.
13. **Richards, H. M.** Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. Jahrb. Wiss. Bot. **30**: 665–688. 1897.
14. **Steinberg, R. A.** A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. Amer. Jour. Bot. **6**: 330–372. 1919.
15. **Stevens, F. L., and Hall, J. G.** Variation of fungi due to environment. Ann. Rept. N. C. Agr. Exp. Sta. (1909) **32**: 47–71. 1911.

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1922

President: HENRY C. COWLES
University of Chicago

Vice-President: MARGARET C. FERGUSON
Wellesley College

Secretary: I. F. LEWIS
University of Virginia, University, Va.

Treasurer: IRVING W. BAILEY
Bussey Institution, Harvard University

Councilors:

N. L. BRITTON
New York Botanical Garden

J. C. ARTHUR
Purdue University

C. E. ALLEN
University of Wisconsin

Any one actively interested in botanical work is eligible for membership. Candidates for membership must be recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including one subscription to the AMERICAN JOURNAL OF BOTANY. Candidates proposed for membership, and approved by the Council, may receive one copy of the JOURNAL at $4.00 per year pending their election at the winter meeting of the Society.

The Little Things That Count Big In Owning Your Own Greenhouse

Ever gone into a hat store, knowing exactly the kind and shaped hat you want; and then have some smart Alec of a salesman try to sell you the kind HE likes?

Makes a fellow madder than a wet hen, doesn't it?

Suppose, however, that same salesman had told you something about some of his hats that meant better service to you—more satisfaction for your money—you then sort of feel towards that salesman as you would an old friend.

Salesman number two is our sort when it comes to selling a greenhouse.

We do sell greenhouses, but we first sell satisfaction.

Catalog for the asking.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

PHILADELPHIA
133 So. 12th St.

Vol. IX. NOVEMBER, 1922 No. 9

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

CONTENTS

The structure of the starch grain . . . . O. L. Sponsler 471
A study of the white heart-rot of locust, caused by Trametes robiniophila . . . . C. H. Kauffman and H. M. Kerber 493
The origin and development of lamellae in Agaricus campestris and in certain species of Coprinus . . . . Michael Levine 509

Smithsonian Institution
DEC - 1 1922
National Museum

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
At Prince and Lemon Sts., Lancaster, Pa.
LONDON AGENTS
WHELDON AND WESLEY, Ltd.
2, 3, & 4 Arthur St., London, W. C. 2

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
The Thompson Institute For Plant Research, Inc.

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

H. H. BARTLETT,
University of Michigan

ARTHUR H. GRAVES,
Brooklyn Botanic Garden

I. F. LEWIS,
University of Virginia

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Postage to Canada, 20 cents; to other foreign countries (except Mexico and Cuba) 40 cents a volume on annual subscriptions. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume, post free.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $4.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or The New Era Printing Co., Inc., Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

Vol. IX November, 1922 No. 9

THE STRUCTURE OF THE STARCH GRAIN

O. L. Sponsler

(Received for publication January 23, 1922)

Although starch is one of the most common and most widely distributed, as well as one of the most widely used, of organic substances, there is amazingly little known about it. During the past two centuries fully five hundred investigators have worked with starch, and yet there are only a few points which seem to be at all well established. In 1836, Poggendorff (1), in his review of the work done on starch, wrote that "no substance has been investigated more and is still less known"; Carl Nägeli (2) in 1858, Arthur Meyer (3) in 1895, and E. T. Reichert (4) in 1913, in their large monographs express a similar view; and a reviewer of 1921 would very probably arrive at the same conclusion.

One commonly thinks of starch as a product of corn, wheat, and other cereals; or of the potato tuber, the cassava root, or the stem of the sago palm; and recognizes it as a product of plant life, and as the principal form in which food is stored.

Commercially it is separated by grinding the plant tissues in which it is stored and allowing it to settle out from a water mixture. When washed and dried the white powdery mass is the starch of commerce.

Under the microscope this powder is seen to consist of minute, transparent, glossy beads, varying in size from 1/10 of a millimeter down to the limit of visibility, that is, about 4/10,000 of a millimeter in diameter. They vary also in shape, although the general tendency is towards a rounded, approximately spherical form. On close examination in water they appear to consist of a series of more or less concentric layers, or lamellae. In polarized light a dark cross appears in each grain. If, however, the grains are made to swell by treating them with hot water, or with certain chemicals, the dark cross disappears.

The natural whole grains seem to be insoluble in cold water; but if treated with hot water or if the individual grains are crushed, they swell and appear to go partly into solution. The air-dried grains usually contain a high percentage of moisture, often up to 20 percent.

[The **Journal** for October (9: 415–469) was issued October 20, 1922.]

Problems Presented by Starch

These statements hint at a few problems which in turn lead to many more. At this place, however, there is no need to do more than to give briefly the status of the larger groups of these problems, in order to locate, in the whole field of the work on starch, the position of that on the structure of the grain.

About the first problem which attracted the early investigators was that concerning the identity of the components, or compounds, which make up the starch grain. Starting with Leeuwenhoek (5) in 1719, many investigators have attempted to solve the problem, but up to the present time, the scores of contradictory papers on the subject lead one to conclude that the correct answer is not yet firmly established. Many methods have been tried and repeated, but there is still considerable uncertainty whether starch consists of a single substance or of two or more substances. Perhaps it is the ease with which starch becomes modified that is the cause of so much uncertainty.

The place of origin of the starch grain is more certain. It has been verified many times and can be considered as settled. Crüger (6, p. 46) in 1854 pointed out that all starch grains originate in the protoplasm of the cell; Schimper (7) in 1880 reported his discovery of the unstable albuminous bodies which always accompany starch grains; and Strasburger (8) a few years later put on a firm basis the statement of the earlier workers, that starch grains are formed inside plastids (protoplasmic bodies)—chloroplasts in the green parts and leucoplasts in those parts which are not green. He reports cases, however, in which these bodies are not differentiated from the rest of the protoplasm.

The mechanism of the formation of the grain is not known. Only one thing seems certain; that is, that the grains grow only where they are in contact with plastids.

Many of the chemical and physical properties have not yet become fully established. About the only one which seems quite satisfactory to the chemists is the percentage composition. They have accepted $C_6H_{10}O_5$ as reported by Guérin Varry (9) in 1834, but whether a dozen or a hundred of those groups constitute a molecule is not at all certain. The formula, therefore, is written $(C_6H_{10}O_5)n$, and the molecular weight is often written "n 162.1." The solubility of starch is uncertain and the specific gravity is variable. The color reaction with iodine solution varies greatly with the conditions of the experiment. The reactions with acids and enzyms lead into a large field of uncertainties, which, however, have little or no direct bearing on the present work.

Structure of the Grain

The physical structure of the grain has been a subject of much discussion during the past hundred years; and since this paper deals with one of the many problems involved in determining the physical structure, this phase will be described in considerable detail.

The grain appears to have a layered structure somewhat analogous to that of an onion, that is, sphere inside of sphere. Strictly speaking, however, the more truly spherical layers occur only near the center; the other layers are generally thicker on one side, making the shape of the larger grains eccentric.

One group of investigators, working primarily with swollen grains, considered the grain as a sac composed of a different substance from the enclosed starch. The lamellar structure was more or less ignored. Leeuwenhoek (5) and Raspail (10) were among the earlier members of this group, and Whymper (11) and Beijerinck (12) are among the present-day representatives.

A smaller group report evidence sufficient to warrant the supposition that there is a series of concentric vesicles representing layers of the grain. Maschke (13) W. Baily (14), and Mme. Z. Gatin-Gruezewska (15) were advocates of this theory.

By far the greater number of investigators have considered the layers as essential parts of the structure of the grain. That the layers differ in composition was held by Maschke (16), Nägeli (17), Meyer (18), Kraemer (19), and Gatin-Gruezewska (15). That they differ physically, perhaps dense and less dense layers alternating, was favored by Fritsche (21), Münter (22), Kabsch (23), Sachs (24), Strasburger (25), and de Vries (26).

The possibility of a crystalline structure of the grain was first brought out by Raspail (27) in 1825; but on account of its existence fully formed and free in plant cells, of its glossy round form, its insolubility in water, its color reaction with iodine, and its decolorization with alkali, he decided it could not be a crystal structure.

Münter (28) considered that he had proved the grains to be crystalline, although they could not be recovered in crystalline form, when he discovered that they would dissolve in sulphuric acid and that the solution would turn blue with iodine.

Whatever these investigators may have had in mind concerning the structure of the crystal, it remained for Nägeli (2) in 1858 to develop the first theory which was to hold a large place in the literature on the minute structure of the grain. He conceived the layers to be composed of invisible particles, and developed this theory along with his intussusception hypothesis of the growth of the starch grain in his monograph "Die Stärkekörner." It is a rather complex theory in that there are many assumptions concerning both the particles and the tensions and forces involved. In brief, the theory is as follows (2, pp. 332–377): The layers of the grain are built up of small particles of starch, the largest of which is invisible with the most powerful microscope, yet it contains over 9000 molecules of starch. These particles, which he later calls micellae (29), are surrounded by a water-shell which is thin on the larger particles and thick on the smaller particles. They may consist of either of the two substances, granulose or cellulose, which Nägeli considered to be the two components of starch. The denser layers of the grain are composed of the larger particles while the less dense or softer layers contain only the smaller particles with the thick water-shell.

He supports this construction by long discussions of the forces which must be exerted to account for various starch reactions with water. In several places he said these particles are not crystals, but in 1881 (29) he stated that the micellae are of a crystalline nature.

About thirty years after Nägeli's theory appeared, Schimper (30) undertook to determine whether the starch grain is crystalline or amorphous. He decided that the cohesive and the optical properties would differentiate these two states. From his work with polarized light he concluded that the grains must consist of fibrous crystals arranged at right angles to the concentric layers; and that they differ from the ordinary spherocrystal through their ability to swell in water.

In 1895, Arthur Meyer (3) in his large monograph, "Untersuchungen über die Stärkekörner," accepted, to a great extent, the conclusions of Schimper and elaborated the spherocrystal conception of the starch grain. He used the definition (3, pp. 101–107) of the spherocrystal which Nägeli (31) and Rosenbusch (32) proposed—that it is a more or less globular body composed of radially arranged needle-like crystals. Fox Talbot (33) in 1836, Sir David Brewster (34) in 1853, Rosenbusch (32) in 1885, and others had studied the optical properties of globular crystal aggregates in which they could see the needle-like crystals arranged radially from a central point. The dark cross produced by polarized light seemed to be the same in them as that formed in other globular bodies in which the individual crystals were invisible. The latter, then, they reasoned, consisted likewise of invisible radially arranged crystals. In addition to this, they found spherical forms which presented gradual transitions from those with visible crystals to those in which the crystals were not visible.

Basing the conclusion primarily on this work, Meyer (3, pp. 116–129) felt justified in assuming that the starch granule is a spherocrystal. The invisible needle-like crystals he called trichites. He decided that the concentric layers of the grain are visible because the trichites differ in size and number in the different layers. By comparing the properties of inulin and amylodextrin spherocrystals with those of the starch grain he arrived at the following conclusions:

1. In the starch grain radial lines of weakness occur just as they do in the spherocrystals, where they are due to radially arranged trichites.

2. Optically the spherical grains behave exactly like the spherocrystal, while the eccentric forms behave as though they were built up of trichites placed at right angles to the concentric layers of the grain. Very small broken pieces also behave as though composed of trichites.

3. The concentric layers in the spherocrystals are visible because the space between the trichites varies. This might be the cause in the starch grain also.

4. The layers are due to periodic changes in the mother-liquor, which affects the growth and form of the trichite in both the spherocrystals and the starch grains.

These comparisons and conclusions, based to a great extent upon experiment, led Meyer to conclude that the grain is a spherocrystal. Only a few investigators have opposed this theory; many have accepted it.

H. Fischer (35) found the dark-cross phenomenon in hardened mucilage of certain orchids, and Strecker (36) found the same effect in the guard cells of the stoma. Czapek (37) considered both of these cases to be due to symmetrically distributed tensions and inferred that the dark cross of the starch grain might be due to similar causes.

Reichert (4, p. 82) in his large monograph on starch accepts the spherocrystal conception without question.

Perhaps the latest bit of evidence in this connection is from work done with X-rays. Herzog and Jancke (38) reported a crystalline structure for both cellulose and starch, although no details of their work with starch were given. The method they used is one by which it is possible to determine whether a powdered substance has an amorphous or a crystalline structure; also, if the latter, to determine the crystal system, although in certain systems the problem becomes very complex. Further, in many instances it is possible to determine the location of the different kinds of atoms which make up the crystal.

That method is the one used for the experimental work which was done for this paper, and is described in detail below.

THE X-RAY METHOD OF DETERMINING CRYSTAL STRUCTURE

The method consists, briefly, in photographing the X-rays which are reflected from the various atomic planes of a crystal. In order to explain this method it may be of advantage to have some of the concepts which will be used described in terms of present-day science.

The Crystal

Since 1850, the face of a crystal has been considered an indication of its internal atomic structure (39). No direct evidence of this was brought forward until Laue (40) in 1912 conceived the idea that if X-rays are actually electromagnetic waves, they should show interference phenomena when reflected from a crystal, since the size of the waves approaches the magnitude of the distance between the atoms of a crystal. He worked out the mathematical part of the problem, and Friedrich and Knipping (41) proved experimentally the correctness of his figures. From this brilliant beginning the structure of many crystals has been determined; resulting in the present-day concept of a crystal as an arrangement of atoms so placed that all of them lie in planes which are regularly spaced. The cubical crystal of common table salt will serve as an illustration. One could think of the structure of such a crystal as somewhat like that of a cubical box exactly filled with equal-sized oranges, each orange representing an atom. The oranges would arrange themselves in layers, and no matter whether the box were standing on a flat side or balanced on an edge or on a corner, there would be, horizontally, layer above layer. When the box is standing with a flat side on the floor, the distance between the center planes of the layers will be, say, 1 unit. If the box is balanced on an edge, the distance between the new horizontal planes will be less than the unit; actually it will be $\frac{1}{2}\sqrt{2}$ (or .707) times the unit. If the box is balanced on a corner, then the planes which are now horizontal will be still closer together, actually $\frac{1}{3}\sqrt{3}$ (or .577) times the unit. In each case the distance between the atoms of a single plane becomes greater. That is, as the planes become closer together the atoms in the planes become farther apart. There are many other planes, but there is no need of further discussion of them here. Of course, the atoms may be relatively farther apart than the oranges in the illustration, leaving considerable space between them. One can visualize this arrangement as a sort of lattice with the atoms at the corners, a three-dimensional lattice. The expression "space lattice" is commonly used in this kind of work.

In mineralogy there are several ways of designating the planes described above; the system generally used in the X-ray work with crystals designates the first plane as the 100 plane, the second as 110, and the third as 111. These figures refer to a relation between the planes and the axes of the crystal. More detail concerning the crystal is probably not necessary here.

X-Rays

The modern conception of the atom is that it consists of a core, or nucleus, surrounded by a system of electrons, which are situated at a considerable distance, relatively, from the core. The electrons may be arranged in concentric rings, the number of both electrons and rings varying with the different elements. One or perhaps several of these may be shot off from the atom by appropriate means without changing the elemental nature of the atom.

If a high-voltage electric current is allowed to pass through a vacuum tube which has at its cathode a fine wire spiral at white heat, a stream of electrons will be shot off from the spiral to the anticathode. They will attain a velocity somewhat less than that of light. If this stream of electrons is allowed to impinge on a metal anticathode, the atoms of that metal will be jarred into vibration as the electrons hit them. This vibration starts a series of waves in the surrounding ether, from each atom. These waves are X-ray waves. The wave-lengths are of the order of the Ångström unit (1 Å. (Ångström) = $\frac{1}{10,000,000}$ mm.). Every metal produces more or less of all wave-lengths. These can form a spectrum comparable to that of white light. Further, each metal produces a few wave-lengths which are characteristic of itself and which have an intensity far in excess of the others; just as in light, each element has its characteristic lines. The characteristic wave-lengths produced by a rhodium anticathode, such as was used in the experimental work which is to be described below, are .617 Å. and .533 Å. in length. The energy conveyed by these waves is not carried in equal amounts by all wave-lengths, usually one or two carrying almost all of it. Of those from rhodium the .617 Å. wave-lengths carry perhaps 70 percent of the total characteristic ray energy, while the .533 Å. wave-lengths carry perhaps more than half of the remainder.

A beam of such waves, when reflected from a crystal, will be sorted out into a spectrum of several lines which may be caught on a photographic plate. The line due to the .617 Å. wave-lengths will be much darker than the others, and is called the α line. The .533 Å. line, called the β line, will be much less intense, and a third line, the γ line, will be still fainter. The first two are all that are of importance here. In fact, the lines obtained in the present set of experiments are all α lines, with the exception of a few faint β lines. The "white light" or general radiation, as it is sometimes called, merely causes a general darkening of the negative. It follows that the beam, for purposes of explanation and for coarse work, may be considered as consisting of only the .617 Å. wave-length.

The statement that the waves are "reflected" is not quite true, but the end results are comparable to those of light reflection so that the idea is conveyed by the word. A light wave, which may be 10,000 times the length of an X-ray wave, will be wholly reflected from a layer of atoms such as the surface layer of a crystal, while the short X-ray wave will be only partly reflected; in fact, only a very small part is reflected from a single plane. By far the larger part passes through to the next plane, where a minute portion is again taken from it and reflected, and the remainder passes on to the next plane beneath, continuing in this way for perhaps a million planes each reflecting only a minute portion.

The so-called reflection is brought about in this way: when a wave hits an atom it sets the atom to vibrating, and that atom in turn produces a secondary set of waves of the same kind. These secondary waves from a plane of atoms will form a wave front which will leave the plane at the same angle at which the primary beam strikes it; that is, the angle of incidence is equal to the angle of reflection. It is readily demonstrated geometrically that these secondary waves, produced by planes of atoms, will reinforce each other, resulting in a strong wave front when a certain relation exists between the wave-length, the distance between the planes of atoms, and the glancing angle of the primary beam. This demonstration was made and is clearly explained by Bragg and Bragg (45). The relation which is fundamental in all work of this sort is expressed by the equation

$$\lambda = 2d \sin \theta,$$

where λ is the wave-length, d is the distance between the planes of atoms and θ is the angle between the planes and the beam of X-rays. From these relations it follows at once that where only one wave-length and only one set of planes is used, the crystal will produce "reflections" only when in very definite positions.

These points become clearer perhaps with a little study of figure 1.

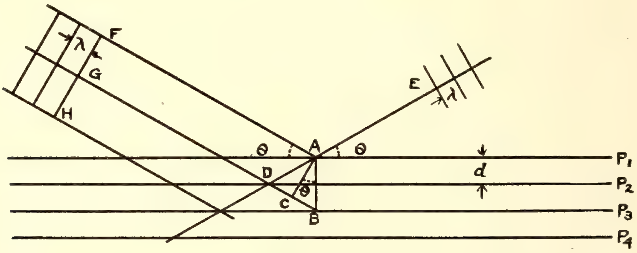


FIG. 1. From "X-ray and crystal structure," by W. H. Bragg and W. L. Bragg.

If P_1, P_2, P_3, P_4 represent the planes of a crystal, planes perpendicular to the paper, and FGH a wave front moving towards these planes, a part of the wave front reflected from plane P_1 follows the path AE and a part reflected from P_2 will likewise fall into the path AE extended from D, and similarly from plane P_3, etc. Now in order to have the wave from P_2 reinforce that from P_1, *i.e.*, to have crest coincide with crest, the wave from D must be exactly 1 wave-length, λ, behind that from A. In other words, the part of the wave from F must travel through a distance 1 wave-length less than the part from G, or

$$FA + AE \text{ must equal } GD + DE - \lambda.$$

Without going into the details of the proof, it happens that CB is the difference between the distances traveled by F and G to reach E; and CB then, if the waves reinforce each other, must be equal to λ. It can easily be shown that the angle opposite CB is equal to the angle θ formed by the beam and the planes, and since $AB = 2d$, then,

$$CB = AB \sin \theta,$$
or
$$\lambda = 2d \sin \theta.$$

If the difference between the distances traveled by F and G to reach E is exactly equal to λ, then the wave from P_1 will be reinforced not only by that from P_2, but also by the waves from P_{1000}, and from any other plane the rays can reach. But if that distance is greater or less than λ, by even one part in 2000, for example, then the wave from P_{1000} will neutralize that from P_1; and the one from P_{1001} will neutralize that from P_2, and so on. The neutralizing effect is not quite complete, because the incident rays reaching P_{1000} are weakened by passing through so many planes. So it happens that unless λ is almost exactly equal to $2d \sin \theta$, reflection will fail to occur. It is important to notice that this conclusion is based on the assumption that the reflecting body consists of a large number of planes. If, on the other hand, the number of planes from which reflection occurs is small, then the neutralizing effect becomes less complete and the reflected line becomes broad and blurred; and an unusually large range of angles will be effective in producing the reflected line.

Reflection from Crystal Planes

In discussing the structure of the crystal a few pages back, three prominent sets of planes were mentioned. Those parallel to the faces of the cube, which were called the 100 planes, were separated by a uniform distance d. If that crystal were placed so that a narrow beam of X-rays hit these faces making the glancing angle equal to θ_1, then a reinforced train of waves would be reflected from them. If it were placed so that the angle were slightly greater than $2\theta_1$, another reinforced train of waves would be reflected, and the equations would be

$$\begin{aligned} \lambda &= 2d \sin \theta_1, \\ 2\lambda &= 2d \sin \theta_2, \\ 3\lambda &= 2d \sin \theta_3, \text{ etc.} \end{aligned}$$

These are reflections of the first order, second order, third order, respectively. The subscript figure is used to designate the larger angles and incidentally the order of reflection. The second-order lines are very weak, and those of the third order are still weaker. The latter are not considered in the present work.

If the crystal were placed so that the beam made the proper glancing angle with the 110 set of planes, then again a reinforced reflection would occur. The distance between these planes is $\frac{1}{2}\sqrt{2}$ times the distance between the 100 planes, so that now d has a new value, and since λ remains the same, $\sin \theta$ must be a new value in the equation, $\lambda = 2d \sin \theta$. Another change in values takes place when the 111 planes are in position to reflect the beam, and again d and θ would have new values. The equations would read for the first-order reflections:

$$\begin{aligned} &\text{For 100 planes, } \lambda = (2d \times 1) \sin \theta_1'. \\ &\text{For 110 planes, } \lambda = (2d \times .707) \sin \theta_1''. \\ &\text{For 111 planes, } \lambda = (2d \times .577) \sin \theta_1'''. \end{aligned}$$

The accent marks indicate different values of the angles which produce first-order reflections.

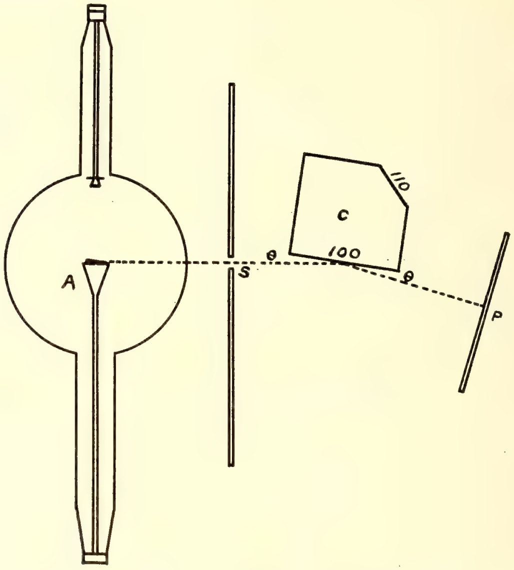


FIG. 2. X-ray tube with crystal in position.

A diagram of an X-ray tube with a crystal in position (fig. 2) may make this somewhat clearer. *A* represents the anticathode which is the source of the X-rays. *S* is a lead screen which absorbs all the rays except a beam which a slit allows to pass through. This beam is incident on the crystal *C* from which the reinforced waves are reflected to the photographic plate *P*, where they produce an effect resulting in a black line when the plate is developed. In the position shown, the rays are incident on the 100 planes. If, without disturbing the tube and the plate, the crystal was moved so that the glancing angle θ would be increased the proper amount, then a line of the second order would be produced from the same planes. If still further changes were made, without disturbing the tube and the photographic plate, the two orders of reflection from each of the two other planes, 110 and 111, would be thrown on the plate, one at a time. Since each reflection has a definite glancing angle which is different from the others, there would be produced on the plate six lines.

It seems perfectly plausible that six small crystals could be put in place of the one large crystal, *C*, and be so arranged that one would produce the first-order reflection from the 100 planes; another one so that it would produce the second-order reflection from the same planes; a third so that it would produce the 110 first order, and a fourth the second order; the remaining two could produce the first and second orders from the 111 planes. With this arrangement all six lines could be photographed at the same time.

It is only a step further to break those six crystals into many smaller crystals, since the crystal structure cannot be destroyed by that means, and to pack them in a small tube of some amorphous substance, like glass or celluloid; to allow the beam of X-rays to pass through this tube; and to photograph the many minute reflections which will be produced by this random arrangement of tiny crystals. Since all of those small crystals which have their 100 planes at the proper angle of incidence to the beam will send their reflections out at the same angle, the sum of these small reflections will make a large line on the photographic plate; this will happen also with each of the other planes for both first- and second-order reflections; and as a result the photographic plate will have all six lines which might have been obtained by carefully manipulating a single large crystal or by carefully arranging six smaller crystals.

From this it is just another step further in thought to realize that a powder of an unknown structure might be substituted for the known powder, and certain facts concerning its atomic structure be obtained from the photographic plate.

Based, more or less, upon this sort of reasoning, Hull (42) and Debye and Scherrer (43) independently devised methods, which are very much alike, for studying the atomic structure of crystal powders. Since then several modifications have been made in the refinement of the apparatus, but the essential features have remained the same. They also determined certain conditions which must be complied with for the success of the method, such as the most suitable X-ray tube, and the best voltage, connected with the production of the rays; the fineness of the powder which will give the clearest lines, and the kind of containers for the powder. Through their work and that of others, means have been found for obtaining almost pure monochromatic beams, that is, one wave-length only; and for increasing the effect on the photographic film by converting the waves which have passed through the film into light waves.

The experimental part of the work for this paper was done with an apparatus built on the principles laid down, for the greater part, by Hull (42, 46).

Experimental Part

Apparatus

The apparatus (fig. 3) consists of two parts: an X-ray tube (its high-voltage electrical equipment not shown) and the photographic part. The X-ray tube, *X*, is enclosed in a lead-covered box, *B*. The photographic film, *F*, is placed on the circumference of a flat, semicylindrical film-holder, *H*. The powder to be investigated is pressed into a container, *P*, which is located at the intersection of all the radii from the film. The beam of X-rays from the anticathode, *A*, passes through two adjustable lead slits, *S*, *S*, and into the powder where a small part of the beam is reflected to the film at various definite angles while the larger part passes through the powder and is absorbed by the lead shield, *L*, just before it would otherwise reach the film. Concerning the details of construction only enough will be

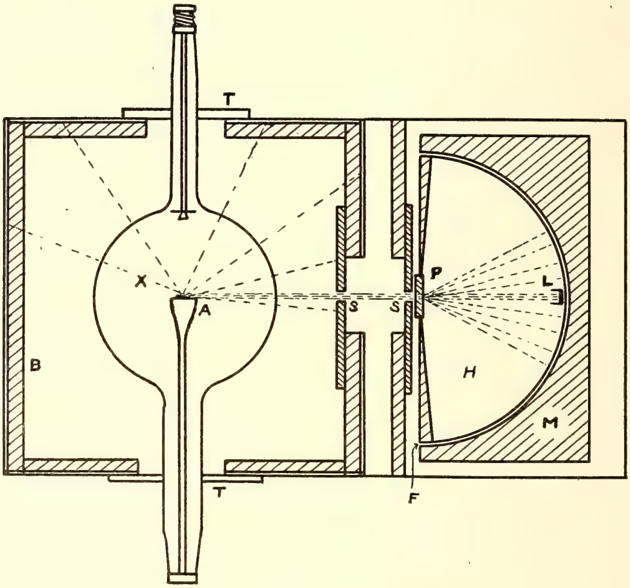


FIG. 3. X-ray tube and film-holder viewed from above.

described to supplement figure 3 in making the experimental work clear. Several detailed descriptions, which vary only slightly, are in the literature (42, 43, 44, 46).

A Coolidge tube with a broad-focus rhodium anticathode was used as the source of the X-rays. The bulb of the tube is 18 cm. in diameter. The box which incloses the tube is approximately 30 cm. on each side and is covered with sheet lead 2 mm. thick. The tube is supported by sheets of asbestos, *T*, *T*. The lead slides, *S*, *S*, are about 2 cm. high and adjustable to form a slit of any width.

The film-holder, *H*, is a hollow, semicylindrical box 5 cm. deep with a radius of 14 cm. The block, *M*, which fits around it is of solid wood and also 5 cm. deep. The film lies between these two parts. The film-holder, *H*, is made from three semicircular pieces of sheet lead. One forms the top, one the bottom, and the third divides the box into halves, an upper and a lower. They are held in place on the straight side by two pieces of wood and on the curved side by the lead shield, *L*, which is about 5 mm. thick. Without this absorbing shield the film would become heavily fogged at this place. The whole of the curved side of the holder is covered with black paper.

The container for the powder is merely a piece of sheet lead, about 3 mm. thick, with two windows cut through it, one above the other, into which the powder is pressed just firmly enough to support itself so that no covers are needed. When in position (at *P*) each window opens into one compartment of the holder and so provides for making photographs of two powders at the same time.

When the apparatus is ready for exposure, the two adjustable slits are opened only enough to allow a narrow, ribbon-like beam of X-rays to pass through. This beam is about 1 to 2 mm. wide and 20 mm. high. When it strikes the powder container, the upper half passes through the powder in the upper window and the lower half through the lower window. The part of the beam which goes straight through strikes the lead shield, *L*, and is absorbed; while the part which is reflected by the tiny crystals meets but little resistance in the black paper and so reaches the film. The rays which react on the film fall into two categories: the first contains the reflected characteristic rays which form the lines; the second contains those rays which produce the general darkening of the film between the lines. The rays of the second category are in part reflected "white light," or general radiation, and in part from any amorphous material present; both will cause a diffuse scattering like that produced on light by smoke or fog. Rays of the first category only can produce the lines and so indicate the presence of crystalline matter. The lines will be approximately the width of the lead slits, *S*, *S*.

In this type of film-holder the primary beam cuts the film into halves which, so far as the lines are concerned, are mirror images. There are at least two advantages arising from this: (1) since the lines must appear in each half, the possibility is eliminated of mistaking a developer flow line; and (2) the error in measuring the arc of the glancing angle is reduced, since the distance between pairs of similar lines is four times the length of the arc.

The electrical part of the apparatus was calibrated so that the voltage could be kept at approximately 40,000 volts and the current at from 3 to 5 milliamperes.

The powder for examination was ground fine enough to pass through a sieve having 200 meshes per inch. It was found that if the particles were too large to go through such a sieve, the lines produced on the photographic film were composed of large spots; but if the particles are small enough to pass through, the spots are invisible and the lines appear solid.

Each sample was pressed into the lead slide with just enough pressure to make a tablet-like mass which would be solid enough to support its own weight. The thickness of this tablet would vary with the substance used, for there is a fairly definite relation between the atomic weights of the elements in a substance and the absorption of X-rays by it. For example, the elements in salt are almost twice as heavy as those in starch, so the tablet of starch which would allow the maximum amount of reflected energy to pass through to the photographic film would be approximately twice as thick as the most efficient salt tablet.

Eastman's duplitized X-ray films were used, two films being used for each exposure. The two films made it possible to observe very faint lines which would not be distinguishable on a single film.

Test Reflections

In order to test the apparatus, sodium chloride was used first, for its crystal structure has already been worked out and it is known to produce lines readily by this method. An exposure of 4 hours, at 40,000 volts and 4 milliamperes current, produced the lines shown in figure 4.

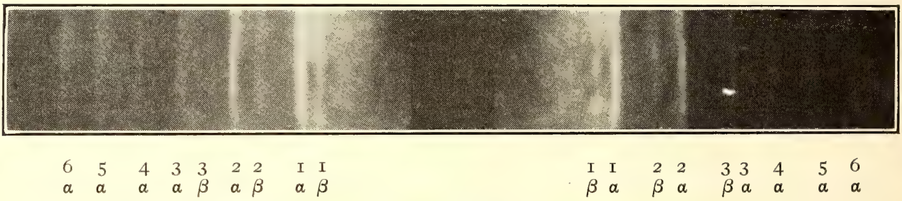


FIG. 4. Sodium chloride, positive print. Reflected lines are light.

If these lines corresponded favorably with their calculated position, then the apparatus could be expected to produce lines from other crystal powders. Also the denser lines, those numbered 1_α, 2_α, etc., should correspond with the theoretical position of the lines made by reinforcement of the waves having a .617 Å. wave-length; and the fainter lines, numbered 1_β, 2_β, 3_β, should be in the positions where reinforcement occurs of the waves having a .533 Å. wave-length. The latter carry much less energy and would therefore make fainter lines.

Tables 1 and 2 give the results of measurements of the glancing angles, and of the computations based on the equation, $\lambda = 2d \sin \theta$, which shows

TABLE 1. *Lines Produced by Sodium-chloride Powder; Rhodium Anticathode;* $\lambda = .617$ Å.

No. of Line / α Lines	Distance between Lines (mm.)	Glancing Angle θ*	Sin θ	Distance between Atomic Planes $d = \frac{1}{2}\left(\frac{\lambda}{\sin \theta}\right)$	d Calculated	Ratio	Indices of Planes
1	61.5	6° 18′	.10973	2.81 Å.	. . . Å.	1.00	100
2	87	8° 54′	.15471	1.99	1.99	.707	110
3	107	10° 57′	.18995	1.63	1.62	.577	111
4	122.5	12° 32.5′	.21715	1.41	1.41	.500	100†
5	138	14° 7′	.24390	1.26	1.26	.447	210
6	152	15° 34′	.26836	1.15	1.15	.408	211

* The film, when in the film-holder, lies on the circumference which subtends all of the glancing angles. By construction, 1° on this circumference equals 2.44 mm.

† This line is the result of reflection of the second order from the 100 planes; and here, *d* should be exactly $\frac{1}{2}d$ as obtained from the reflection of the first order from those planes.

the relation between the wave-length, the distance between planes of atoms, and the glancing angle θ. The tables also give, for comparison, the calculated distances between planes based on the assumption that line 1 was produced by the 100 planes of the crystals.

TABLE 2. *Lines Produced by Sodium-chloride Powder; Rhodium Anticathode;* $\lambda = .533$ Å.

No. of Line β Lines	Distance between Lines (mm.)*	Glancing Angle θ	Sin θ	Distance between Atomic Planes $d = \frac{1}{2}\left(\frac{\lambda}{\sin\theta}\right)$	d Calculated	Ratio	Indices of Planes
1	53	5° 26′	.09469	2.81 Å.	. . . Å.	1.00	100
2	76	7° 47′	.13543	1.96	1.99	.707	110
3	95	9° 35′	.16648	1.60	1.62	.577	111

* These lines are so faint that close measurement is not possible.

Since the actual and calculated values are so close, two inferences may be drawn: first, that any lines produced by another powder, in place of the salt, must be reflected by the same sort of planes, *i.e.*, planes of atoms; and secondly, that the principal lines will be α lines.

It is possible to cut out the β lines completely or practically so by using a filter made from the metal, or a simple salt of the element, which occurs in the periodic table one or two places below the metal used in the anticathode. In the case of a rhodium anticathode the filter would be made of ruthenium. But the filter reduces the intensity of the lines perhaps 50 percent, and this would mean a considerable increase in the time of exposure. Since, however, the salt photograph shows that the principal lines are the α lines, the preliminary work can be carried on without using a filter.

There is supposed to be a relation between the intensity of the secondary waves produced by an atom and its atomic weight. On this account, it was thought necessary to try a carbohydrate known to be crystalline, such as cane sugar, in order to determine the best conditions for getting reflections from carbon and oxygen atoms. The upper half of figure 5 shows

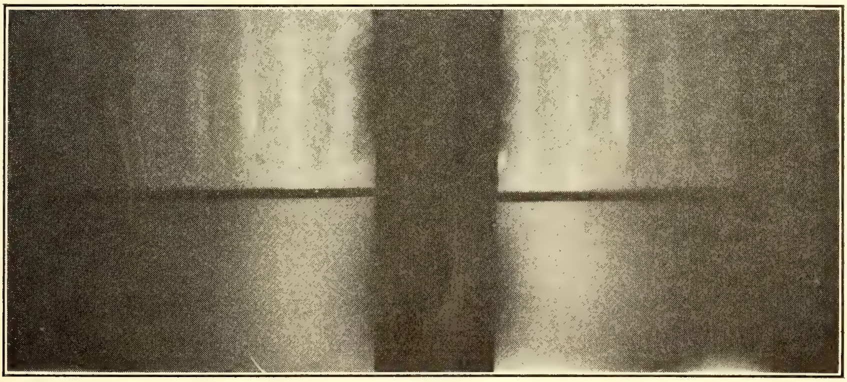


FIG. 5. Above, cane sugar; below, dextrine.

the results obtained, and it seemed from this that any other carbohydrate in which there was uniform atomic arrangement would also give lines due to reinforcement of the reflected waves. If, however, the atoms were not uniformly arranged, lines should not appear, and in order to demonstrate this, another carbohydrate, dextrin, which is generally considered amorphous, was photographed along with the cane sugar. An exposure of 18 hours was given. The lower half of figure 5 shows the picture obtained from this amorphous substance, in which the rays are merely scattered, not reinforced at any single place. The atoms in air give the same sort of scattering, but much less intense, as shown in figure 6, with an exposure of 18 hours.

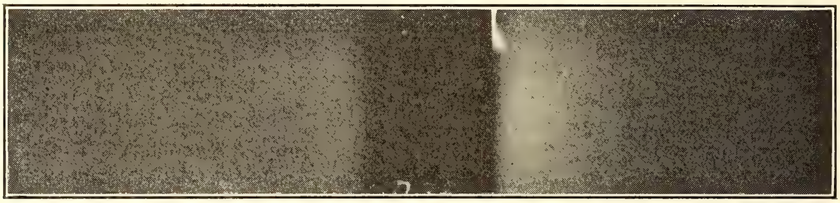


FIG. 6. Air.

Reflections from Starch Grains

After these preliminaries, in which it was shown that the apparatus would produce lines with a crystalline, and none with an amorphous, substance; that the principal lines were α lines; and that the exposure for

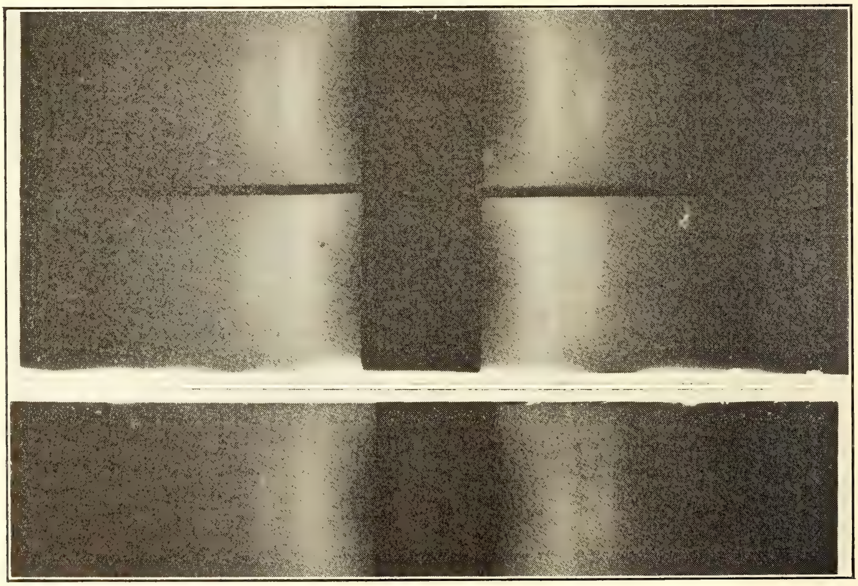


FIG. 7. Above, potato starch; middle, cassava starch; below, corn starch.

carbon and oxygen atoms was about 18 hours; three different kinds of starch were photographed: corn, potato, and cassava.

The dry powder consisting of whole grains of starch was pressed into the specimen-holder just firmly enough to support itself. Potato and cassava starch were taken at one time; and corn starch and a blank at another time. The exposures were for 18 hours. The voltage was approximately 40,000, and the current varied between 3 and 5 milliamperes. Figure 7 shows the results of these exposures. The lines are very much blurred, and on that account are difficult to measure. Careful study brought out several more lines on the films than are shown in the prints made from them.

In tables 3, 4, and 5 the measurements and the computed results are given. The computations are based on the equation, $\lambda = 2d \sin \theta$, where $\lambda = .617$ Å., d is the distance between planes, and θ is the glancing angle determined from its arc which was measured on the photographic film.

TABLE 3. *Corn Starch*

No. of Line	Sin θ	d Actual	d Calculated from Cube	Difference	Indices of Planes of Cube
1*a*	.05175	5.96 Å.	5.87 Å.	−.09	$111^{(\times 2)}$
1	.06210	5.04	. . .	. . . .	100
2	.08020	3.85	3.56	−.29	110
3	.10366	2.98	2.91	−.07	111
4	.11667	2.64	2.52	−.12	$100^{(2)}$
5	.13399	2.30	2.25	−.05	210
6	.14608	2.10	2.06	−.04	211
7	.15845	1.95	1.78	−.17	$110^{(2)}$

TABLE 4. *Cassava Starch*

No. of Line	Sin θ	d Actual	d Calculated from Cube	Difference	Indices of Planes of Cube
1	.06076	5.08 Å.	. . . Å.	. . . .	100
2	.08020	3.85	3.59	−.26	110
3	.10366	2.98	2.93	−.05	111
4	.11494	2.69	2.54	−.15	$100^{(2)}$
5	.13053	2.36	2.27	−.09	210

TABLE 5. *Potato Starch*

No. of Line	Sin θ	d Actual	d Calculated from Cube	Difference	Indices of Planes of Cube
1	.05902	5.20 Å.	. . . Å.	. . . .	100
2	.07962	3.88	3.68	−.20	110
3	.10366	2.98	3.00	+.02	111
4	.11667	2.64	2.60	−.04	$100^{(2)}$
5	.13139	2.35	2.32	−.03	210
6	.14608	2.10	2.12	+.02	211

Before any conclusions are drawn from the tables it must be understood, first, that the lines from no. 3 to no. 7 are so dim that their measurements must be rather uncertain, and secondly, that the error in measurement is large because of the width of the lines and of their blurred condition. How large this error may be is shown by several sets of measurements given in table 6, taken under very favorable conditions of light and at different times, four to six measurements of each starch.

TABLE 6

Distances between Planes for Line 1

Corn starch varied from	4.99 to 5.08	Average, 5.04
Cassava starch varied from	5.03 to 5.15	" 5.08
Potato starch varied from	5.15 to 5.22	" 5.20

Distances between Planes for Line 2

Corn starch, all measurements		Average, 3.85
Cassava starch, all measurements		" 3.85
Potato starch varied from	3.85 to 3.93	" 3.88

Compared to the figures for the salt measurements given above, these variations are large. But in spite of this fact, there are certain rather constant variations which seem to make the tables worth a few moments' study.

In the first place, there is a marked similarity between the three kinds of starch, also between the starches and the calculated spacings for a cubic arrangement, as shown in table 7.

TABLE 7. *Comparison of d for the Starches and for a Cube Based on the Average for Line 1*

Line No.	1	2	3	4	5
Corn	5.04	3.85	2.98	2.64	2.30
Cassava	5.08	3.85	2.98	2.69	2.36
Potato	5.20	3.88	2.98	2.64	2.35
Cube based on ave. of line 1	5.11	3.62	2.95	2.56	2.29

On the other hand, it is quite certain that there is no nice arrangement of the planes such as in the salt or the sugar crystals. Both the blurring of the lines and the irregular spacing are evidence of that.

In column 5 of tables 3, 4, and 5 are recorded the differences between the actual *d* and the theoretical *d*. Line 2 shows a consistently large difference. Line 4 also shows a large difference, although less than line 2. This leads one to suspect that the arrangement is not quite cubic. The blurring of the lines, however, makes the error in measurement too large to warrant further speculation in that direction. Finer and sharper lines are needed before going further.

Another point which determines certain future work is brought out by the no. 1 lines of corn starch. Here the broad line is resolved into two lines, 1 and 1*a*, while with potato and cassava starches only one broad line is visible. Refinement of the apparatus will very probably bring out lines which will suggest a different arrangement of atoms than the cubic as assumed up to this point. On the other hand, accepting the evidence as it exists now, line 1*a* may be the β line ($\lambda = .533$ Å.) for the 100 planes, just as line 1 is the α line for those planes. If that assumption is correct, then d in the equation, $\lambda = 2d \sin \theta$, should be the same for both wavelengths. Actually, line 1*a*, using $\lambda = .533$, gives d as 5.02, while line 1, using $\lambda = .617$, gives d as 5.04. The two values for d agree very closely, but with the range of error so large, nothing more than mere suggestion is safe.

There is another possible significance to the line 1*a* which, if verified, may lead deeper into the composition of the layers of atoms. The position of this line is very close to the place where the reflection from the 111 planes would occur if these planes were alternately composed of different kinds of atoms—for example, of layers of carbon atoms alternating with mixed layers of hydrogen and oxygen. Here, again, more refined apparatus is needed.

Quite another set of speculations is started from another photograph. If the starch grain were built up of minute crystals as A. Meyer (3, pp. 116–129) contended, then a mass of crushed grains ought to produce the same lines that the whole grains do. In order to demonstrate the truth or fallacy of this contention, dry potato starch was ground in a pebble mill until samples contained no whole grains. Figure 8 shows the result of photographing, at the same time, the reflections from the crushed grains and from the whole grains. A check photograph gave the same results. Careful study of the films failed to reveal any lines produced by the crushed starch. It does not seem probable that the crystalline structure could be changed into an amorphous form by mere crushing. To test that assumption, cane sugar was ground in an agate mortar until the size of the minute

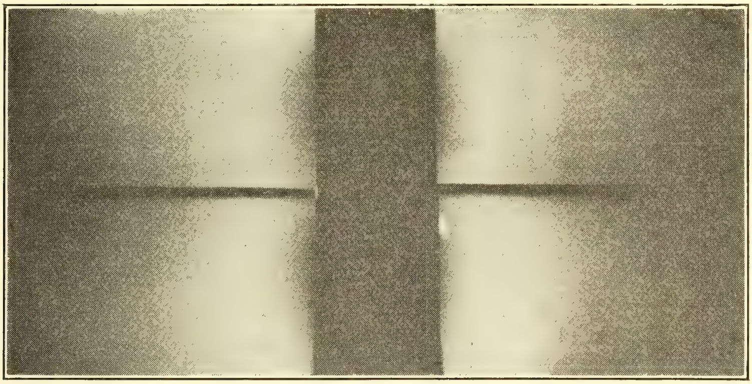


FIG. 8. Above, potato starch, whole grains; below, crushed grains.

particles agreed favorably with the size of the crushed starch particles when examined under the microscope. Figure 9 shows the resulting photograph. The lines are fully as strong and even clearer in this, and the exposure was fully 20 percent less than in the previous cane-sugar picture (fig. 5). The first and obvious conclusion concerning the starch is that the lines produced by the whole grains are due to the structure of the grain. The second conclusion is that the structure is neither crystalline nor amorphous.

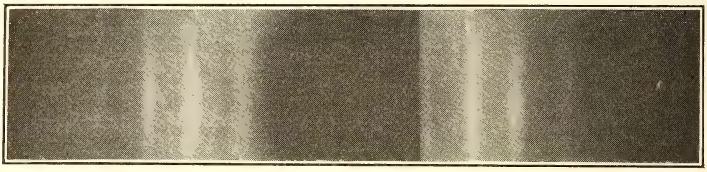


FIG. 9. Finely pulverized cane sugar.

Before speculating further on a possible structure of the whole grain, it is interesting and suggestive to compare with this work on starch some recent work done on wood and cotton cellulose fibers. In 1920, Herzog and Jancke (38) published a table of figures which they obtained by exposing wood and cotton cellulose to X-rays, in a similar manner to that which has just been described for starch. They published only the values of (sin θ)/2. These have been converted into ratios, and are given in table 8 along with those for starch.

TABLE 8. *Comparison of Ratios of Atomic Spacings for Cellulose and Starch*

Cellulose			Computed Values of Cube	Starch		
Cotton	Ramie	Wood		Potato	Cassava	Corn
1.00	1.00	1.00	1.00	1.00	1.00	1.00
.670	.677	.682	.707	.745	.758	.765
.557	.558	.554	.577	.575	.587	.592
.486	.499	.496	.500	.507	.530	.524
.443	.448	.443	.447	.452	.465	.456
.366	.367	.372	.408	.404		.416
.339	.346	.343	.354			.387

One of the very few well-established facts about starch is that it is formed only when in contact with a protoplasmic body. Likewise, cell walls are formed only when in contact with protoplasm. Cellulose and starch have long been recognized as being chemically very closely related. Here in table 8 appears again evidence of this close relationship; also there appears a very consistent difference, all the values for starch being above the corresponding ones for a cubical arrangement, while all those for cellulose are below. This would indicate a slight difference in the atomic arrangement. The fact that both are formed at the contact surface of protoplasm favors the idea that the layers of atoms are parallel to the surface, whether curved or plane.

In conclusion, two points seem to stand out as fairly definitely proved: (1) that there is, within limits, a regular and fairly uniform arrangement of atoms in the starch grain; and (2) that this regularity is destroyed by crushing the grain, which leads to the additional statement that the regularity is not that of crystalline structure. These statements oppose the spherocrystal theory of Schimper (30) and Meyer (3), while they favor the assumption made by Kabsch (23) in 1863 that the grains have a physical uniformity which, however, is not sufficiently regular to assign crystallographic axes to them. Or it may be that, instead of planes such as are found in crystals, the regularity takes the form of curved layers.

It is a pleasure to record here an indebtedness to Dr. George J. Pierce of the Department of Botany, and to Dr. David L. Webster and Dr. Frank C. Hoyt of the Department of Physics, for helpful assistance and suggestions.

Stanford University

BIBLIOGRAPHY

1. **Poggendorff, J. C.** Annalen Phys. Chem. **37**: 114–160. 1836.
2. **Nägeli, C.** Die Stärkekörner. Pp. 624, 16 plates. Zürich, 1858.
3. **Meyer, A.** Untersuchungen über die Stärkekörner. Pp. 318, 9 plates. Jena, 1895.
4. **Reichert, E. T.** The differentiation and specificity of starches, pts. I and II. Pp. 1250, 102 plates. Carnegie Inst. Wash. Pub. 173. 1913.
5. **Leeuwenhoek, A.** Opera omnia, vol. 4. Epistolae physiologicae . . . Delphis, apud Adrianum Beman, p. 232. 1719. (Quoted by Payen in Ann. Sci. Nat. Bot. II, **10** : 8, 1838.)
6. **Crüger, H.** Bot. Zeit. **12**: 41–50. 1854.
7. **Schimper, A. F. W.** Bot. Zeit. **52**: 884–886. 1880.
8. **Strasburger, E.** Über den Bau und das Wachstum der Zellhäute, pp. 154–159. Jena, 1882.
9. **Guérin Varry.** Annales Chim. Phys. II, **56**: 225–252. 1834.
10. **Raspail, F. V.** Ann. Sci. Nat. I, **6**: 384–427. 1825.
11. **Whymper, R.** 7th international congress of applied chemistry, sect. VI*a*, pp. 7–11. 1909.
12. **Beijerinck, M. W.** Kon. Akad. Wetensch. Amsterdam, Proc. Sect. Sci. **14**: 1107–1110. 1912.
13. **Maschke, O.** Jour. Prakt. Chem. **56**: 403. 1852.
14. **Baily, W.** Philos. Mag. V, **2**: 123–126. 1876.
15. **Gatin-Gruezewska, Z.** Compt. Rend. Acad. Sci. Paris **146**: 540–542. 1908.
16. **Maschke, O.** Jour. Prakt. Chem. **56**: 409. 1852.
17. **Nägeli, C.** Die Stärkekörner, p. 181. Zürich, 1858.
18. **Meyer, A.** Untersuchungen über die Stärkekörner, pp. 242–246. Jena, 1895.
19. **Kraemer, H.** Bot. Gaz. **34**: 352. 1902.
21. **Fritsche, J.** Ann. Phys. Chem. **32**: 131. 1834.
22. **Münter, J.** Bot. Zeit. **3**: 198. 1845.
23. **Kabsch, W.** Zeitschr. Anal. Chem. **2**: 216, 217. 1863.
24. **Sachs, J.** Textbook of botany (Eng. transl.), pp. 57, 58. Oxford, 1875.

25. **Strasburger, E.** Über den Bau und das Wachstum der Zellhäute, p. 162. Jena, 1882.
26. **de Vries, H.** (Abstr.) Bot. Jahresber. **13**^{1}: 122. 1885.
27. **Raspail, F. V.** Ann. Sci. Nat. I, **6**: 387, 388. 1825.
28. **Münter, J.** Bot. Zeit. **3**: 194, 195. 1845.
29. **Nägeli, C.** Bot. Zeit. **39**: 637, 638. 1881.
30. **Schimper, A. F. W.** Bot. Zeit. **39**: 223–226. 1881.
31. **Nägeli, C.** Sitzungsber. Bayer. Akad. Wiss. **8**: 206. 1862.
32. **Rosenbusch, H.** Mikroskopische Physiographie der petrographisch wichtigen Mineralen, 2d ed., pp. 33, 65. Stuttgart, 1885.
33. **Fox Talbot.** Trans. Roy. Soc. Edinburgh **20**: 607. 1853. (Quoted by Brewster.)
34. **Brewster, D.** Trans. Roy. Soc. Edinburgh **20**: 607. 1853.
35. **Fischer, H.** Beitr. Biol. Pflanz. **8**: 74. 1898.
36. **Strecker.** Unpublished statement quoted by Czapek. Biochemie der Pflanzen, 2d ed. **1**: 402. Jena, 1913.
37. **Czapek, F.** Biochemie der Pflanzen, 2d ed. **1**: 402. 1913.
38. **Herzog, R. O., and Jancke, W.** Zeitschr. Physik **3**^{3}: 196, 197. 1920.
39. **Bravais.** Quoted by Laue. Ann. Physik **41**: 971. 1913.
40. **Laue, M.** Ann. Physik **41**: 971–978, 989–1002. 1913.
41. **Friedrich, W., and Knipping, P.** Ann. Physik **41**: 978–988. 1913.
42. **Hull, A. W.** Phys. Rev. **10**: 661–696. 1917.
43. **Debye, P., and Scherrer, P.** Phys. Zeitschr. **17**: 277–282. 1916.
44. **Bain, E. C.** Chem. Metallurg. Engin., Oct. 5, 1921, pp. 657–664.
45. **Bragg, W. H., and Bragg, W. L.** X-rays and crystal structure. Pp. 230. London, 1918.
46. **Hull, A. W.** Phys. Rev. **17**: 571–574. 1921.

A STUDY OF THE WHITE HEART-ROT OF LOCUST, CAUSED BY *TRAMETES ROBINIOPHILA*

C. H. Kauffman and H. M. Kerber

(Received for publication February 24, 1922)

Introduction

The actual distribution of the active mycelium during the process of decay in living trunks of various timber trees, and the process of such decay in the production of "heart-rots," is still poorly understood. If one examines the literature on the subject, it soon becomes evident, however, that sufficient data are at hand to conclude that the relation of any specific heart-rotting fungus to a specific tree is either specific itself or belongs to one of a group of type reactions. We are far enough in the study to realize that generalizations on these reactions are impossible.

In the study of a rot caused by *Trametes robiniophila* Murr., we paid little attention to the number of trees affected or to the question of the value of locust timber. The inroads of the locust borer, and, in the more southern distribution of the locust tree, the abundant heart-rot due to *Fomes rimosus*, both loom as so much more important enemies to be controlled before the locust tree can find its rightful place in lumber economy, that it seems futile to attempt to make an important case of the rot in question.

Our attention was therefore directed to an analysis of one case: the macroscopic and microscopic characteristics of the rot; its mode of advance; the distribution of the mycelium in the various portions of the affected trunk; and its effect on the various elements of the wood.

That the white heart-rot of *Robinia Pseudo-Acacia* L. is quite frequent in southern Michigan there is no doubt. Its presence is not to be reckoned by the number of sporophores observed, since it appears to fruit sparsely, and when it does the sporophore rarely lasts more than one season or is soon attacked by insect enemies or disintegrated by wind and weather. The black-locust tree is found scattered along some of the Ann Arbor streets, and not a few show symptoms of heart-rot. From year to year sporophores may be noticed on some of them, and the tree selected for study had had a large yellowish sporophore on it the year before, and at the time of cutting had produced another from an old frost crack that had almost healed over; in addition, signs of several old scars left by former sporophores were still distinguishable on the trunk. Although the tree was suffering from both borer attacks and the heart-rot, as shown by a number of large dead branches and the straggly condition of the crown, its leaves were green and vigorous, and in general still making considerable growth. The internal condition of the trunk and dead branches would soon, however, have made it a prey to wind and storm. This tree was about 35 years old and had reached a height of 31 feet with a 24-inch base.

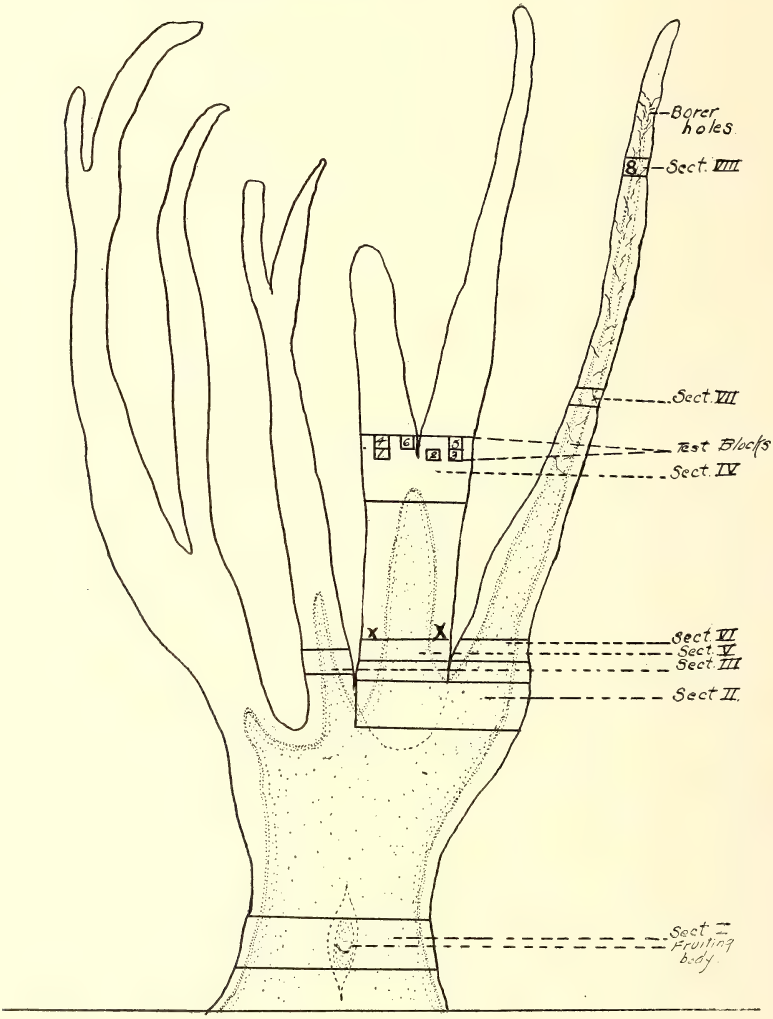

Borer holes
8
Sect. VIII
Sect. VII
4
7
6
2
5
3
Test Blocks
Sect. IV
X
X
Sect. VI
Sect. V
Sect. III
Sect. II.
Sect. I
Fruiting body.

FIG. 1. Diagram showing diseased area of the black locust. (Infection by *Trametes robiniophila* Murr.)

After cutting and sectioning, it was soon evident that the fungus had entered by an old borer hole through one of the large upper branches now dead. As shown in the diagram of the tree (fig. 1), the rot, starting in this branch about 15 feet above ground, moved downwards through the branch and trunk. Another main branch near by had in the meantime become affected upwards from the trunk only a short distance. Both these branches were perforated with borer channels horizontally and vertically, and when present, either in apparently sound wood or in slightly rotten wood, these channels would be found to contain mycelium. The spread of the rot from these channels is shown in figure 2.

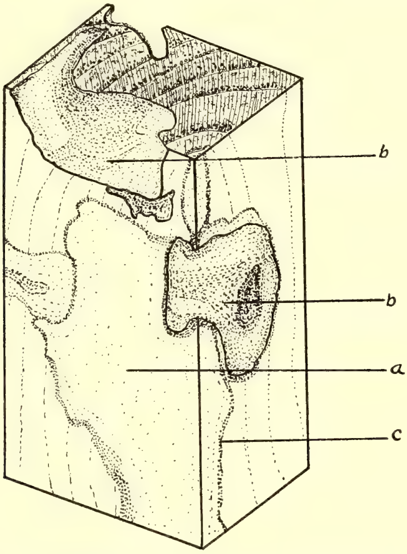


FIG. 2. From a piece of diseased black locust, cut from the region of the sound wood of section VI. It is clearly shown that the points of infection throughout the diseased tree were the borer channels. *a*, area of decay; *b*, borer channels; *c*, dark border zone of rot.

Old borer channels in badly decayed wood were indistinct and were recognized by pellets of dead mycelium.

DESCRIPTION OF THE ROT

The diseased area of rot of *Trametes robiniophila* may be divided into three stages:

1. *Black Border Zone.* The apparently sound wood of the tree is sharply differentiated from the rotten area by a very fine, distinct, brownish-black zone, having a width that varies between 1/4 and 1/2 millimeter. This zone is very irregular in outline, but invariably tends to maintain the same color and width.

2. *Lesser Decayed Portion.* There is a sharp differentiation between the apparently sound and the decayed wood immediately adjoining and outside the black zone. The decayed wood is of a light fawn color to a brownish white except where traversed by borer channels where it assumes a dark brown to reddish appearance. The borer channels are filled with old, dead mycelium. The decayed wood is of a solid texture, but very much softer and lighter in weight than the sound wood. This first stage of rotted wood cuts very easily without breaking.

3. *The Last Stage of Decayed Wood.* The color of the most badly decayed wood remains the same as that of the less decayed stuff, but there is a difference in its texture. Instead of the wood being soft and easily cut without breaking, it has now become very dry and friable and is easily crumbled between the fingers. It cannot be cut without breaking. In addition, white streaks appear at frequent intervals in the vertical sections, and upon examination these are found to be pure wood fibers, containing very little lignin. These streaks are present in only the very final decay of the tree. The borer channels are not visible, being choked with old masses of mycelium which closely resemble in color the decayed wood. The contents of these channels have a rubbery consistency and are in the form of pellets. The mass of decay holds together fairly well, but would crumble away in time and thus cause a hollow interior.

The rotted portion of a section taken near the base appeared like a star in outline, or as if some powerful acid had been spilled over the section and had changed the texture of all the wood with which it had come into contact. The dark border ring was not very distinct in this section horizontally, but could be seen very well on the longitudinal portion. At a few points, the fungus became powerful enough to break through the sapwood and cambium in order to form fruiting bodies, and here the decayed wood appears a darker brown, of soft, friable texture.

Microscopic Characteristics of Fungus and Host Tissue

The tree was cut up and eight sections were selected for study. These are shown in the diagram of the tree (fig. 1).

Before much work was done on the actual course of the fungous hyphae in the cells, quite a bit of time was spent in the laboratory in preparing free-hand sections of sound wood, partially decayed wood, and badly decayed wood. These sections were both radial and tangential and were taken from section I (see fig. 1). These sections were stained with chloriodide of zinc for cellulose reactions and with phloroglucin and hydrochloric acid for lignin reactions. These tests were later confirmed by the use of eosin and also of congo red, the latter as a cellulose test. The best test for cellulose and lignin, aside from chloriodide of zinc, and phloroglucin and HCl, was that of haematoxylin for cellulose and safranin for lignin. In the case of the haematoxylin, the Heidenhain method was the best.[1] Finally, in order to compare the cellulose and lignin tests in a positive way, microtome sections were made of wood from a sound tree from another source, cut in the three planes: cross, radial, and tangential. There was, of course, quite an elaborate technique involved in merely trying to find the differentiation of cellulose and lignin tissue in black locust, but the time was well spent as will be shown later. Permanent slides were attempted for the diseased portion of the wood of the tree studied but without success, because of the damaging influence of the hydrofluoric acid on the already weakened tissues.

---

[1] The sections were washed in distilled water and then immersed in a 2 percent solution of iron alum. They were left in the iron alum for about five minutes and then washed twice in distilled water, care being taken to get all the free alum out of the sections before transferring them to the haematoxylin. When this had been accomplished, the sections were transferred to a 1/2 percent solution of haematoxylin and allowed to remain for a minute or two. They were again washed and then transferred to a 1 percent solution of safranin, made up of equal parts of alcohol-soluble and water-soluble safranin. The result was a blue for the cellulose and a red for the lignin. The sections can be left for any length of time in the safranin.

All sections were taken from sound wood of section I and from one to two inches in from the sapwood. Sections were radial and tangential ones. These tests were corroborated by permanent mounts of black locust stained with haematoxylin and safranin.

TABLE I. *The Result of Staining for Cellulose and Lignin in the Normal Wood*

Elements	Cellulose Test	Lignin Test
Med. ray cells	Good cellulose test, deep blue color; not all ray cells showed cellulose test	Some ray cells showed good lignin color (True of ray cells in heartwood of black locust)
Tracheids and vessels	Did not show any cellulose reactions, with the exception of the tyloses in the vessels, which are pure cellulose	Showed a good lignin test, with exception of tyloses
Wood parenchyma	These showed a good cellulose blue reaction, in decided contrast to the vessels which they surround	Did not show a lignin test
Wood fibers	These showed both a cellulose and lignin reaction, due to two types of fibers present in locust: mucilaginous fibers which stain blue with haematoxylin, and the ordinary fiber which stains red	Showed a good lignin stain for the ordinary wood fiber present in black locust

In testing the decayed portion of section I, sections were taken of (*a*) the black border zone; (*b*) the less decayed portion of the wood; and (*c*) the badly decayed portion of the wood. Radial and tangential sections were made of all these parts.

TABLE 2*a*. *Black Border Zone of Decay; Results of Testing the Tissues for Cellulose and Lignin. Width of Zone, 1/4 to 1/2 mm.*

Elements	Cellulose Test	Lignin Test
Med. ray cells	Good cellulose test, but does not show through brown area	Slight lignin test as observed in sound wood
Tracheids and vessels	No cellulose test	Good lignin test
Wood parenchyma	Good cellulose test where not hidden by brown substance	No lignin
Wood fibers	Cellulose for mucilaginous fibers	Good lignin test for ordinary wood fiber

TABLE 2*b*. *Lesser Decay of Wood; Results of Testing the Decayed Tissue for Cellulose and Lignin. Sections made Free-hand from Larger Section I*

Elements	Cellulose Test	Lignin Test
Med. ray cells	Good blue color for ray cells	No lignin remaining in ray cells, and where present very faint
Tracheids and vessels	Deep blue color in tyloses	Fair lignin, but not as deep red as that in normal wood
Wood parenchyma	Fair bluish stain with haematoxylin	No lignin present
Wood fibers	Still bluish for mucilaginous fibers	Fair lignin present

TABLE 2*c*. *Badly Decayed Portion of Wood; Results of Testing the Decayed Tissue for Cellulose and Lignin. Sections both Radial and Tangential, Taken from Section I*

Elements	Cellulose Test	Lignin Test
Med. ray cells	Very good blue color	No lignin present
Tracheids and vessels	Those which are still remaining give a good blue for the tyloses	Scarcely perceptible lignin color
Wood parenchyma	Give good reaction for cellulose	No lignin
Wood fibers	Color is absent in the ordinary fibers and blue is present in the mucilaginous fibers	Give a very faint to colorless reaction with a lignin test

The results thus speak for themselves. The hyphae have the power of dissolving the lignin from the wood by enzymatic action.

Study of the Elements of the Normal Black Locust

Black locust normally has very little sapwood, this in most cases being only three to four rings in width. This sapwood is of a whitish to a pale lemon color and is in decided contrast to the golden brown of the heartwood. It is a ring-porous hardwood, that is, there is a distinct variation in the size of the spring line of vessels as compared to those of the summer wood, as seen in cross section. These vessels in the heartwood are filled with tyloses, which fact makes locust one of the choice woods for tight cooperage stock. The tyloses are also supposed to act as a protection against the invasion of disease. The vessels are of the very highest types, having simple end-wall pits and spiral vessel markings. The tracheids also have spiral wall thickenings, but these are of the tertiary type. It may be mentioned in this connection that the tertiary thickenings of the spiral walls of the tracheids resemble the hyphae of *Trametes robiniophila* so closely that no attempt was made to find hyphae in the tracheids.

Wood parenchyma is found only surrounding the vessels in *Robinia Pseudo-Acacia*. This fact marks the wood as vasicentric wood—one of the highest of the hardwoods. The wood-parenchyma tissues are cellulose and stand out clearly when a haematoxylin stain is used. The cell walls are comparatively thin, and the pits are simple when communicating with the ray cells.

The fibers are of two kinds:

1. *Libriform Fibers*. These are called mucilaginous fibers in black locust. Jeffrey says (The anatomy of woody plants, pp. 33, 34):

> In these elements the inner portion of the wall has become more or less completely modified into a mucilaginous state which causes it to stain strongly with haematoxylin. The presence of fibers of this description is often of value from the hygroscopic quality imparted to the wood, which prevents undue shrinking or swelling. These numerous mucilaginous fibers made the wood particularly valuable for tree-nails in the days of the construction of wooden ships.

2. The second kind of fiber present in black locust is the ordinary wood fiber, with a thick wall composed of lignin. These fibers are the principal source of strength, hardness, and toughness in broad-leaf woods. Their function is principally a mechanical one. In Robinia, they are in rather large, compact masses in the late wood, separated by groups or bands of pores and parenchyma.

The ray cells and wood parenchyma are the only living tissues in the heartwood of the locust, and even these too become lignified after a time. Lignification of the ray cells is common in the early stages of heartwood transformation. The ray cells of locust belong to the diffuse type of ray, for they have more than one layer of ray cells. They are filled with living tissue, mostly containing starch, and give a cellulose reaction with the proper stain. The ray cells are storage cells for food and help in the distribution of water in the horizontal plane of the tree. The ray cells have simple pits where they communicate with the wood parenchyma, and semi-bordered pits where they communicate with the vascular tissues, such as the tracheids and vessels.

Diseased Wood

Both macerated material and free-hand sections of the different stages of the rot were used. By this means data were obtained as to the exact rôle played by each kind of element in the advancement or retardation of the mycelium in the wood. For maceration, the pieces were placed in a solution containing 10 percent nitric acid and 10 percent chromic acid in equal proportions. The containers were kept in a warm bath for 2¼ hours, then washed clear of the acid and kept in water with a few drops of chloroform. The free-hand sections were stained with eosin in order to bring out the hyphae when present. The following results were brought out by these studies:

1. *Black Border or Transition Zone.* Here was noticed a distinct, narrow, dark-brown strip of rot, whose elements were infiltrated for the most part by a brownish substance. This circular strip was varied by small, V-shaped irregularities, the point of the V taking its origin in the medullary ray and wood-parenchyma cells. These two kinds of elements also contained the darkest infiltrations, especially the ray cells as observed in tangential section. The base of the V-shaped irregularities encompassed the tracheids, vessels, and both kinds of wood fibers. The contents of the last named were much lighter in color, and seemed to indicate greater resistance on the part of these elements to the dissolving action of the hyphae. No hyphae were observed in this zone, but evidence of their former presence was plentiful; the ray cells and wood parenchyma showed the characteristic small, rounded perforations, and some of the pits of the tracheids and vessels were eroded. All kinds of solvents and stains were tried to change the color of the brown deposit, but without success. In general it may be said that little damage seemed to have been caused by the hyphae, for the elements seemed perfectly normal except for the brown and blackish inclusions and the holes made by the hyphae in the walls. This zone, as far as its woody elements are concerned, is in about the same stage of decay as the apparently sound zone beyond it, which is to be discussed later. If hyphae were present, they must have been overlooked because of the optical difficulties in the study of this zone.

This blackish-brown substance has been variously accounted for. Rhoads (1917) gives a summary of the views held by different workers. That it is a phase in a series of chemical changes taking place within the dead cells seems an unavoidable conclusion, since the zone containing these colored inclusions passes outward as the rotten core advances radially. We did not concern ourselves with the question of its origin, whether an infiltration, a gradual change from original protoplasm and other cell contents, or a residue from wall surfaces. The forward movement of this zone of dark elements is, however, a significant consideration in interpreting this substance. It may not be out of place to speculate, from our advancing knowledge of colloidal chemistry, that, if it is of protoplasmic origin, colloidal stages might be assumed of which the brown color is a character, to be followed by physico-chemical changes through which, by a change from the colloidal condition into molecular constituents, the color disappears. In such processes time is as much a factor as is the necessary ratio of the ingredients. Von Schrenk's (1914) findings in Syringa, according to which the brown substance is supposed to be destroyed by the fungus in its later activities, can play no part in our case. As will be shown later, there is no active mycelium behind the black zone to which we can refer this action. Nor can we say that the black zone is formed by the advance guard of the oncoming hyphae, since, as will also appear below, the hyphae are present and active beyond and outside the black zone.

2. *Lesser Rot Zone.* Inside of the black zone, and by indefinable stages to the very rotten center, we have a zone marked sharply only on its exterior side. Here the decay may be said to be less complete than in the central zone. The hypha-holes through the walls of the elements were much larger and much more numerous than in the black zone. This was particularly true for the ray cells and the wood parenchyma. The cell contents of both ray and wood parenchyma had entirely disappeared. The larger hypha-holes were measured and found to range from three to ten microns in diameter. The other elements were all attacked by the hyphae, the holes being smaller and less numerous. The fibers seemed to be affected least of all. No hyphal threads could be found, although a diligent search was made for them. In a tangential or radial section containing the tracheids it is very easy to mistake the tertiary thickenings of the walls for mycelium, due to the width, color, and direction of these thickenings, and therefore scant attention was paid to the tracheids, the most careful search, however, being made in the wood parenchyma and medullary ray cells. When single macerated cells were teased out and examined, it was found that the holes made by the hyphae were rounded and smooth and much larger than the width of the hyphal threads as found later. This fact may be accounted for by assuming that the fungous enzyms still persisted although the hyphae had disappeared, and hence the diameter of the holes was increasing as solution continued. It was also found that in these isolated cells the hyphae must have entered the cells through the pits, as well as penetrating directly through the wall. The medullary ray cells and wood parenchyma, although badly decayed, did not break up in the same fashion that the vessels and tracheids did. The fibers held together very well. Not a particle of starch or even of living matter could be found in the wood parenchyma or in the ray cells of this portion of the decay.

3. *Badly Decayed Areas.* In the center of the rotten core the wood was

so brittle that good free-hand sections could be made only with great difficulty. An attempt to imbed and make microtome sections failed because of the crumbly nature of this material. It was noticed that the size of the hypha-holes had increased, until in places the walls were so thoroughly riddled that they had parted as soon as the middle lamellae were dissolved by the maceration fluid. All the elements were so affected, with the possible exception of the fibers which still held the wood together, supported by the skeleton cell walls of the rays. The tracheids and vessels were merely a broken mass of fragments and recognizable only through their spiral markings, etc. In both the medullary ray cells and the wood parenchyma, it appears that the hyphae merely utilized the starch and other contents of these cells and then worked through from these cells as origins, to the vessels, tracheids, and fibers. The wood parenchyma surrounds the vessels in Robinia, and therefore this would be a particularly good place for the hyphae to work from. This is also true of the ray cells, which are very closely linked up with the other elements.

In macerated portions of this badly rotted area, it was found that the wood parenchyma and the medullary ray cells, although badly riddled, still retained their original outlines and were still easily recognizable, whereas the tracheids and vessels were recognized only through the fragments that had spiral markings. The fibers in most cases were perforated in scattered areas but still held together strongly.

The very end decay showed nothing but the fibers remaining, and these of a distinctly colorless appearance. The wood parenchyma and medullary ray cells were now not very easily recognized. These fibers were very uneven in outline, indicating that most of the wall had been dissolved away. It is these fibers that give the appearance of white mycelial patches in the very end decay, when the decayed material is placed in water for a few days.

4. *Apparently Sound Wood outside the Black Zone.* Having failed in all our studies to locate mycelium in the rotten portion of the trunk, an examination of the apparently sound wood was undertaken. For this purpose a slab was selected which still had three inches of sound wood outside the black zone, *viz.*, section VII. This was supplemented by section I, with a narrower border of sound wood. Free-hand slices were at first made about two millimeters outside the black zone, and here mycelium was found in abundance. The mycelium was exceedingly fine, from 1 to 1.5 microns in diameter, hyaline, and could easily be confused with edges of walls, etc., except for its characteristic windings and branchings and for the presence of delicate cross walls. Under the eosin stain it assumed a pale greenish color which contrasted sufficiently well with the reddened color of the cell walls to double our assurance. When it occurred in the wood parenchyma as seen in tangential sections, the threads were usually parallel to the long axis of the cell and had branches which penetrated the adjoining

cells either by way of the pits or directly through the cell wall. In the case of the parenchyma cells of the ray, the main threads of mycelium were found traversing the cells crosswise and sending branches into the cell. These hyphae were much more numerous in the medullary and wood parenchyma cells than in the tracheids, vessels, and fibers.

Other sections were then made from portions about 7 cm. beyond the black zone, and stained with eosin. Here also hyphae were present in relatively large numbers. The character of the mycelium could of course be compared with that in the sapwood immediately surrounding the sporophore of *Trametes robiniophila*. At first this comparison was disconcerting, since the hyphae ranged larger as one approached the fruiting body, varying from two to nine microns in diameter. Part of this, however, may have belonged to secondary fungi which had found a foothold in the old and crumbling sporophore or at its base on the dead wood. However, as one examined the more characteristic hyphae here, they were found to have a branching system in which end branches were abundant and became progressively more minute, thus corresponding to the size of the hyphae in the apparently sound wood.

From what was found in these studies it became apparent that, although to all appearance the wood beyond the black zone was normal and exactly like good, sound locust wood, in reality there was no longer any sound normal wood present in this tree in a radial direction from the rotten core, and, from a cursory examination, this was also the case in a vertical direction from the uppermost rot to at least a distance of two feet.

Supporting Evidence of Decay in the Apparently Sound Wood

It seemed reasonable to infer from the presence of mycelium, hypha-holes, and some erosion, even if slight, that the apparently sound wood must already be weakened by this preliminary decay. It was therefore decided to make some tests of the compression strength of this wood so as to be able to make an approximate comparison with the strength of known sound locust wood as given in the tables by Newlin and Wilson (*l.c.*). It is to be understood in advance that the few tests made and the many variables possible in dealing with decayed wood for such tests, can give us no more than a very general idea of the actual difference between such affected wood and sound wood. However, the results were sufficiently contrasting to demand attention.

Two small blocks, about 6-cm. cubes, numbered 1*x* and 2*x*, were cut out of the sound portion of section VI (fig. 1), outside the black zone. Six blocks were cut from the upper portion of section IV (fig. 1), situated vertically above the farthest point of the black zone, being in a portion where advance rot might be expected. The uppermost blocks were numbered 4, 5, and 6. The ones from the next adjoining layer below were numbered 1, 2, and 3. These blocks were situated from 20 to 30 centimeters above the farthest visible rot, which had scarcely entered the lower side of our section IV. As the wood tested had been in the laboratory for about nine months, it had lost considerable of its water content before being tested, a condition, however, which should favor the inferences drawn from the results of the tests.

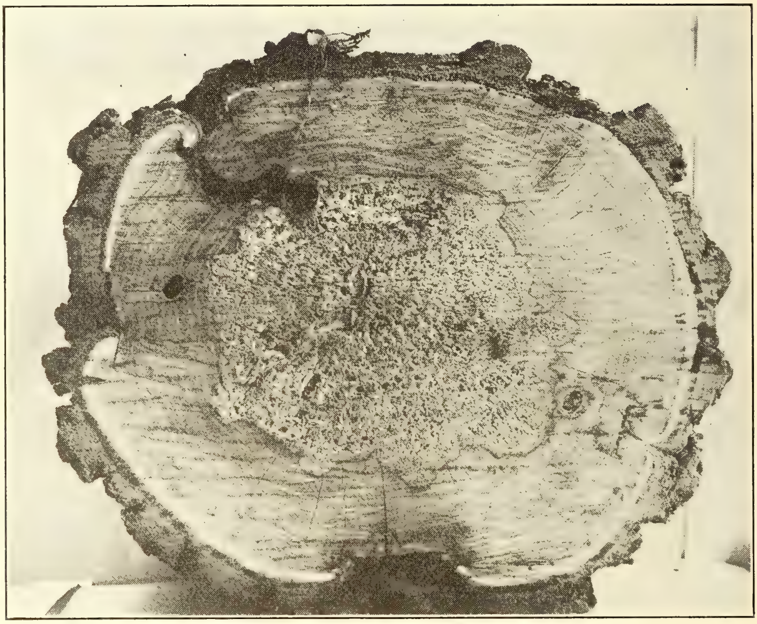


Fig. 3. Cross-sectional view of section VI, showing very clearly the appearance of the rot in the heartwood of the black locust.

Table 3. *Tests of Blocks from Section VI*

Block	Size (Inches)	Crushing Strength			Crushing Strength per Sq. In.		
		1st Fail	2d Fail	3d Fail	1	2	3
1*x*......	1.61 x 1.84	23,000	23,500	24,500	7,770	7,939	8,256
2*x*......	1.6 x 1.33	14,500	15,500	16,500	6,813	7,277	7,746

[The standard of 10,888 lbs. per sq. in. of perfectly sound locust is taken from Newlin and Wilson (1917, p. 40).]

It will be observed that the blocks from section VI had been materially weakened by from 2,000 to 3,000 pounds through the advance of mycelium. The blocks from section IV had been weakened in some cases by half. As is evident at once from a glance through the tables, blocks 1, 2, and 3 all average about the same per square inch when compressed parallel to the

TABLE 4. *Apparently Sound Wood Taken from Section IV*

Blocks 1, 2, and 3, nearest the decayed area; blocks 3, 4, 5 and 6, farthest away from the diseased area.

Block	Size (Inches)	Crushing Strength			Crushing Strength per Sq. In.		
		1st Fail	2d Fail	3d Fail	1	2	3
1.......	1.87 x 1.87	20,000	20,000	20,500	5,714	5,857	5,857
2.......	1.85 x 1.87	19,000	20,000	20,000	5,277	5,555	5,555
3.......	1.80 x 1.80	22,000	22,000	22,000	6,790	6,790	6,790
4.......	1.82 x 1.79	26,500	27,000	27,000	8,134	8,250	8,250
5.......	1.79 x 1.75	29,000	29,000	29,000	9,257	9,257	9,257
6.......	1.83 x 1.80	26,000	26,000	26,000	7,893	7,893	7,893

grain, whereas blocks 4, 5, and 6, which were taken from a greater distance away from the rot, run much higher but still do not approach normal except in the case of block 5, which shows the highest strength per square inch but no resiliency of the wood. In blocks taken from section VI, there is a distinct difference between the first failure and the third failure of about 500–900 pounds, but it should be compared with the test of section IV, in which the failure is final in most cases for a certain load. When the blocks give, they give all at once, which is contrary to what we expect from normal locust wood. It was also noticed in the testing of all the blocks that up to about the final 2/3 pounds per square inch the blocks behaved quite normally, but after heavier weights were applied, the wood reacted in general like one of the softer deciduous woods.

DISCUSSION AND SUMMARY

Several points brought out in this study may prove of far-reaching significance. As already indicated in the remarks about the blackish-brown zone (page 501), certain ideas concerning the occlusions in the elements of this zone must be revised. In this case, at least, these occlusions can play no part as a protection against invading mycelium, as was suggested by Lindroth (1904) and others. Nor can we generalize, as did Rhoads (1917), that "the brown substance ... is indicative of the first stage in the decomposition of the wood"; for the beginnings of the weakening of infected wood, as shown by the tests, are already initiated outside the black zone for a considerable distance, and are probably much more advanced than a microscopic observation alone shows.

"Advance rot" has in recent years been given considerable attention, and during the world war the price paid in human lives as a result of weak spots in aeroplane timber instigated special research along this line by the national government. Unfortunately the results of these studies are not yet available in the literature. Boyce (1920, p. 15) found in the dry rot of incense cedar, where the decayed areas alternate with apparently sound wood, that "hyphae were commonly present in the apparently sound wood surrounding young pockets to a distance of 4 mm., and sparingly to 8 mm. in a horizontal direction," while he found scattered mycelium vertically beyond the last decay pocket to a distance of 7.8 cm. Meinecke (1914) found the advance rot of *Echinodontium tinctorum* on *Abies concolor* to extend vertically from 2 to 6 feet beyond the typical rot. Weir and Hubert (1918), working with the same fungus on *Tsuga heterophylla*, report advance rot recognizable at from 1 to 5 feet. In these cases a trained observer can usually recognize the advance made by the fungus by slight irregularities in color by streaks. Münch (1910) working with a sap-rot, *Stereum purpureum* on poplar, perceived that the advancing hyphae preceded the zone of browning to some extent.

Hartig (1894), speaking of cut timber, suggested long ago that mycelium might be expected in the apparently sound regions of such wood. In the lilac, Von Schrenk (1914) emphasizes the sharpness of the line between "sound" and completely destroyed wood, and all observers know that this is a frequent condition on gross examination. *Fomes igniarius* on poplar, occurring along tamarack swamps in Michigan, shows this contrast most markedly. In other heart-rots like that produced by *Polyporus hispidus* and *Fomes fraxinophilus* on ash, dried-out logs often show a rather indistinct demarcation. But although no generalization can be attempted from the one case studied by us, it would seem that others of the heart-rots found in the hardwoods might be expected to show advance rot, and perhaps to an unsuspected linear extent. The economic importance of this point grows in proportion to the scarcity of sound trees, and the temptation to inspectors of timber to pass slightly decayed stuff is only too well known by our lumber-using manufacturers. If the weakening of the apparently sound wood surrounding a narrow core of rot in a large log of valuable timber were only half what our test figures show, it might still be of serious significance when used for certain structures.

A very interesting academic question remains to be solved in connection with the majority—or at least many—of the heart-rots. What becomes of the mycelium in the rotten core inside the black zone? For years, the senior writer has had his class in forest pathology attempt to locate mycelium in sections of recently cut trees with various heart rots, but in most cases the observations ended negatively, even with most persistent efforts. Mycelium seems to be lacking in most cases in the advanced stage of the rot, or remains only in pockets or cracks as "nests" or "punk," while the rotten tissues seem to be free from it. Hypha-holes and corrosion of the elements in various degrees are easily observed. As noted in detail, this was the situation in the locust tree studied. This, of course, is not true of timber attacked by fungi after cutting, nor apparently of sap-rots, working from without inwards. But even here our data are not very complete. Brown (1915), however, speaking of rot caused by Hymenochaete, says that "in an advanced stage of decay only the openings are left as the hyphae disappear." Boyce (1920) notes that in the incense-cedar rot "hyphae were very rare in the pockets of badly decayed wood," although nests of mycelium occurred in places. He also notes that hyphae must have been present earlier because of the hypha-holes in the cell walls. In the slightly decayed wood of the incense cedar hyphae occur abundantly, although Boyce states elsewhere that "the line of demarcation between sound and decayed wood is very sharp." We have here, then, a situation somewhat different from our case in that hyphae occur back of the line of demarcation, although they disappear later. Von Schrenk and Spaulding (1909) seemed to have little difficulty in finding mycelium of *Fomes igniarius* "in the completely decayed" wood, where, according to these authors, "the mycelium of the fungus is abundant in the large vessels and medullary rays." The hardwood host or hosts which were studied are not mentioned, and it may be presumed—albeit at some risk—that the matter is a variable and that the most frequent condition is not such as they describe. There is, furthermore, no doubt that small masses of hyphae do accumulate in nests or broken-down cavities and there persist, perhaps because of their bulk. The question remains, what happens to the other portions? How do the isolated hyphae, responsible for innumerable holes in cell walls, disappear? Having exhausted the food, they may die and disintegrate. We may say they are resorbed. But this leaves the matter vague, especially as applied to the portions near the line of demarcation between the rot and the apparently sound wood where one would expect dead hyphae to remain visible. The idea of dissolving themselves (resorption) by the ferments of their own making seems unlikely. One other explanation easily comes to mind, *viz.*, that bacteria follow the course of the rot, gaining entrance by the same path as the wound parasite, and destroying the mycelium as soon as the latter has lost its vigor. At first thought this explanation seems plausible. So far, however, no bacteria of wood-destroying ability seem to have been isolated. Schmitz (1919), on the other hand, found strong indications that certain heart-rotting fungi will destroy more wood in the presence of some common and widely distributed bacteria than when these fungi function by themselves. Meanwhile the rotten core seems to be disintegrating still further through the action of some agent. May not the mycelium while still present secrete enough enzyms, which persist actively for a considerable time, and which are responsible for the continued disintegration going on in the rotten core? This whole question still has a strongly unsolved quality and needs further attack.

Although the laboratory and field work was done entirely by Mr. Kerber, the points were worked out by constant coöperation. The authors are under much obligation to Prof. Filibert Roth for the use of the testing machine and for assistance in making the tests; and to Dr. W. W. Tupper for valuable advice and assistance in the study and staining of the locust wood.

Cryptogamic Laboratory,
University of Michigan

LITERATURE CITED

Boyce, J. S. 1920. The dry-rot of incense cedar. U. S. Dept. Agr. Bur. Plant Ind. Bull. 871.

Brown, H. P. 1915. A timber-rot accompanying *Hymenochaete rubiginosa.* (Schrad.) Lév. Mycologia **7**: 1–20.

Hartig, R. 1894. The diseases of trees (Eng. transl.).

Lindroth, J. 1904. Beiträge zur Kenntniss der Zersetzungserscheinungen des Birkenholzes. Naturw. Zeitschr. Forst- u. Landw. **2**: 393–406.

Meinicke, E. P. 1914. Forest tree diseases common in California and Nevada. A manual for field use. U. S. Dept. Agr. For. Serv.

Münch, E. 1910. Versuche über Baumkrankheiten. Naturw. Zeitschr. Forst- u. Landw. **8**: 389–408; 425–447. Über krankhafte Kernbildung. Ibid. **8**: 533–547; 553–569.

Newlin, J. A., and Wilson, T. R. C. 1917. Mechanical properties of woods grown in the United States. U. S. Dept. Agr. Contr. For. Serv. Bull. 556.

Rhoads, A. S. 1917. The black zones formed by wood-destroying fungi. N. Y. State Coll. Forest. Techn. Publ. 8.

Schmitz, H. 1919. Studies in the physiology of the fungi VI. The relation of bacteria to cellulose fermentation induced by fungi, with special reference to the decay of wood. Ann. Mo. Bot. Gard. **6**: 93–136.

Von Schrenk, H. 1914. A trunk disease of the lilac. Ann. Mo. Bot. Gard. **1**: 253–260.

——, **and Spaulding, P.** 1909. Diseases of deciduous forest trees. U. S. Dept. Agr. Bur. Plant Ind. Bull. 149.

Weir, J. R., and Hubert, E. E. 1918. A study of heart-rot in western hemlock. U. S. Dept. Agr. Bur. Plant Ind. Bull. 722.

THE ORIGIN AND DEVELOPMENT OF LAMELLAE IN *AGARICUS CAMPESTRIS* AND IN CERTAIN SPECIES OF COPRINUS[1]

Michael Levine

(Received for publication March 3, 1922)

Introduction

The history of the study of the development of the Basidiomycetes dates back to the earlier part of the nineteenth century. Nees von Esenbeck (1816) should be credited with the description of the origin and development of these plants based on a philosophical conception of how they should develop rather than how they actually develop. Yet many writers, such as Dutrochet (1837), Trog (1837), and others, coupled these notions of Nees von Esenbeck with some actual observation. They were able to distinguish the two essential stages in the life of the mushroom, namely, the vegetative and the reproductive.

Toward the middle of the last century, Schmitz (1842)[2] first described an annular gill cavity separating pileus and stipe in several species of Basidiomycetes and was the first to recognize the veil or cortina. Schmitz conceived a method of development for all pileate fungi in which the organ nearest the substratum in the mature form is the structure first to develop, so that the mycelium is developed first, the stipe second, and the pileus next, the hymenophore being formed last.

Bonorden (1851), working about the same time, was the first to describe the structure of the volva and the manner in which dehiscence is effected. He contributed nothing to the origin of the parts of the plant.

Hoffmann's (1856) observation of the development of the carpophore lies at the basis of many current accounts of the methods of formation of the pileus and hymenium. He described the young buttons of *Agaricus campestris* as small spheres which elongate owing to the growth of the interior cells perpendicularly upward. The terminal cells now grow out laterally and then turn abruptly downward; the ends of these hyphae form the primordia of the lamellae. In 1860 and 1861 Hoffmann described the development of seventeen different species of higher Basidiomycetes. He contends that the fungus first appears in the form of a small white sphere in which a deeply colored central portion of the pileus rudiment

---

[1] The author wishes to express his indebtedness to the Carnegie Institution of Washington for assistance with the publication of the illustrations.

[2] Schmitz studied *Coprinus niveus, Cantharellus sinuosus, C. tubeaformis, Agaricus Bulliardi,* and *Hydnum imbricatum.*

appears. The formation of the gills resembles that of the other angiocarpous forms he described earlier.

De Bary's (1866) work followed soon after Hoffmann's. He described three species[3] of agarics in which he claims to have observed that the young carpophore begins as a mass of delicate and densely interwoven hyphae. Soon this small ball of hyphae becomes divided into two parts by the development of a horizontal annular gill cavity which in median longitudinal section appears as two openings in the upper and inner portion of the undifferentiated hyphal mass. The region lying above a horizontal plane through the gill cavity forms the pileus, while the part below forms the stipe. The layer of hyphae directly above the gill chamber grows into it and forms the lamellae. De Bary's figures of *Coprinus micaceus* in young stages clearly shows the edges of the gills in contact with the stipe, though he does not emphasize their connection with it.

R. Hartig (1874) claims that in *Agaricus* (*Armillaria*) *melleus* the lower surface of the pileus forms an exposed hymenium. A vigorous downward growth of the hyphae sets in from the upper surface and margin of the pileus and a corresponding upward growth of the superficial hyphae of the stipe, thus forming a weft of hyphae or a veil hiding from view the hymenium.

Brefeld (1877) claimed that the carpophore *Anlage* is a hyphal cell that gives rise to a number of branches which intertwine, forming a small mass of coiled hyphae. This mass increases in size, and internal differentiation ensues. The whole carpophore is covered by a loose layer of globular cells which Brefeld held is morphologically equivalent to the volva of the Amanitas and differs only in that in the latter genus the structure is more compact. Brefeld held that the lamellae arise as compact bundles of parallel hyphae each of which has apical growth. Numerous branches are produced which turn to the right and left to form the hymenium. The growth of the lamellae results finally in the adhesion of their edges to the surface of the stipe.

Fayod (1889) contributed his results of the study of a long series of agarics in which he denies Hoffmann's conception of the method of development of the pileus and hymenium. This author maintains that in the upper portion of the spherical button a layer of dense hyphae is differentiated which has the form of an inverted bowl that he calls the *couche piléogène*.

A great number of workers who have studied the sexuality of the Basidiomycetes since the nineteenth century have reported incidentally various phases of the development of these plants, but have not contributed anything of importance to the question of the origin of the gills (see Levine, 1913).

From the time of Fayod nothing was done until the appearance of Atkinson's (1906) work. This took up the question and the manner of origin and development of the carpophore of *Agaricus campestris* vars.

---

[3] *Agaricus campestris*, *A. praecox*, and *Coprinus micaceus*.

Columbia and Alaska. Atkinson claims that the gill rudiments appear first in a longitudinal median section of the young carpophore as two deeply stained areas. These areas represent the cross section of a heavily stained horizontal ring which is the primordium of the hymenium, composed of hyphae which have a very dense protoplasm. The hyphae below this structure rupture, and an annular hollow gill cavity is formed. The lamellae are formed by a downward growth of hyphae from the hymenium primordium into the gill chamber. These observations were confirmed by Atkinson on several other species of Agaricus.

The author of the present paper pointed out (1914) that the origin of the gills in agarics was not yet clearly understood. It was then shown that in the development of the lamellae of *Coprinus micaceus*, the young hymenophore arises at or near the lower surface of the pileus primordium in a manner similar to that described for a number of agarics by Hoffmann (1860), and that it appears in a vertical section of a young carpophore as two densely staining areas of palisade cells, placed to the left and right above the center of the young carpophore.

I pointed out further that the development of this annular primordium of the hymenophore consists in further elongation and, at the same time, specific orientation of these palisade cells. This results in the formation of a series of arched interhyphal spaces or primordia of gill chambers, while the adjacent palisades become the gill rudiments. The point which was emphasized was that the hymenial elements are formed over the arched surface of the interhyphal spaces, rather than on the edges of gill ridges extending downward into an annular gill cavity. In other words, the gill rudiments at this stage are continuous above and below with the fundamental tissues of the stipe and pileus. While De Bary (1887) figured this condition in the development of young buttons of Coprinus, he failed to point out its significance. The report of my observations on *C. micaceus* appeared about the same time as Atkinson's (1914*c*) description of the development of the carpophore of *Amanitopsis vaginata*. No primitive annular gill cavity is formed in this species. The young lamellae extend from the pileus fundament to the stipe region. In this respect the development of Amanitopsis species is like that of Amanita described by De Bary and Brefeld and like that of *Coprinus micaceus* described by me, but, according to Atkinson, is unlike the development of all other agarics. The growth at the margin of the pileus primordium is accompanied by a continuation of the differentiation in the fundamental tissue.

This development in Amanitopsis is identical with the stages found in plants of *Coprinus micaceus* and of other species of Coprinus that I have studied. But for the bulbous base of the stipe region and the thickened volva, sections of Amanitopsis and of Coprinus resemble each other very closely [see figures 83, 92, and 93, Plate XXXIV, and compare them with Atkinson's (1914*c*) figures 10, 13, and 14].

Atkinson reports a primitive annular gill cavity for *Agaricus campestris* (1906), *A. arvensis*, and *A. comtulus* (1914*a*), and *Lepiota clypeolaria* (1914*b*). In *L. clypeolaria* the hymenophore primordium is described as at first smooth. Later, folds are formed which develop into gills. His sections of this material are conspicuous and particularly interesting for the absence of a palisade layer at the time the annular gill cavity is formed (1914*b*, figs. 7–9). In this respect, *L. clypeolaria* is made to differ from all other forms he describes. This appearance is clearly due to the abnormal rupturing of the fundamental tissue below the hymenophore as I am describing it later in this paper.

Prior to 1915, Atkinson classifies the agarics, in which the lamellae arise endogenously, into two groups: those forms in which there is a so-called well-marked annular gill cavity formed below the primordium of the hymenium, as in *A. campestris*, etc., and those in which there is no general gill cavity but rather a series of cavities as in Amanita, Amanitopsis, and in Coprinus described by me. For *Agaricus Rodmani*, Atkinson (1915) finds a new type of annular gill cavity. In this case the general annular gill cavity is interrupted by strands of fundamental tissue which extend from the hymenophore primordium to the stipe region below. These strands are conspicuously attached and are continuous with the tissues of the stipe and pileus and remain attached to the gill fundaments until well-differentiated lamellae are formed. Atkinson is unable to give any adequate explanation of these conditions on his theory that the gill cavity should be annular from the first.

In 1916, Atkinson reports finding a weak prelamellar gill cavity in *Coprinus micaceus* and *C. atramentarius*. In *C. comatus* a well-marked, "strong" annular prelamellar gill cavity is reported. In the same year, Atkinson (1916*b*) reports in *Lepiota cristata* a "weak" annular prelamellar cavity, and in *L. seminuda* a very strongly developed annular gill cavity.

Douglas (1916) studied five species of Cortinarius[4] to determine the origin of lamellae and also the development of the universal veil, but gives no data as to the origin of the gill cavity.

Sawyer (1917*a*) found in *Pholiota squamosa*, *P. flammans*, and *P. adiposa* the appearance of "weak" annular prelamellar gill cavities, although the degree of "weakness" varies in the different individuals. The gills which he finds partially attached to the fundamental tissue become free very late, just before the gills are exposed by the rupturing of the partial veil. Sawyer fails to show the critical stages in the development of the gill salients. His preparations are made of plants too old, and in many cases probably dead, before fixation, although he failed to recognize this fact. His work on *Cortinarius pholideus* (1917*b*) shows nothing further as to the origin of the gill cavity.

Walker (1919) reports on the development of *Pluteus admirabilis* and

---

[4] *Cortinarius distans*, *C. cinnamomeus*, *C. armillatus*, *C. lilacinus*, and *C. infractus*.

Tubaria furfuracea. Although Miss Walker was unable to find stages early in the development of *Pluteus admirabilis*, she contends that in this form the primordium of the hymenophore is exogenous in origin and later becomes endogenous, a claim similar to that made by R. Hartig in 1874 for *Armillaria mellea* which was later corrected by Atkinson (1914*d*).

The studies reported by Atkinson and his students were made on material collected in the field. Specimens were fixed generally with chrom-acetic and picric-acid solutions. In no case so far as reported was the history known of the group of buttons studied. The assumed ages were generally based on size rather than on actual knowledge of the time of appearance of the button.

Adams (1918), in studying the development of *Schizophyllum commune* grown in artificial culture media as well as of plants collected in the field, found, contrary to all observations previously made by other investigators on this form, that the hymenium primordium of this plant arises endogenously. He shows clearly that there is no general annular gill cavity formed into which the young gills develop by downward growth, but that a series of successively formed endogenous gill chambers are produced which become ultimately the spaces between each pair of adjacent gills. These results agree entirely with my observation on the method of origin of the gill cavities in Coprinus. Adams concludes that the gills are essentially hymenium-bearing plates between schizogenously arising gill cavities.

Material and Methods

Since 1913 I have been studying cytologically the development of a number of species of agarics grown in controlled cultures. I have worked especially with *Coprinus ephemerus*, *C. stercorarius*, and *Agaricus campestris* var. *Bohemia* and a white variety possibly *Columbia*, spawn of which was bought from an American spawn producer. The species of Coprinus studied were selected, first, for the ease with which they may be grown under laboratory conditions on their natural substratum as well as on agar; second, for the rapidity with which they grow and develop from spore to mature carpophore; and third, because of the relatively small number of gills. Spores of *Coprinus ephemerus* and of *C. stercorarius* were sown on a variety of agar media. The most favorable was made of a horse-dung decoction. Within seven to ten days after inoculation a great number of young carpophores made their appearance on this medium. These cultures grow best at a temperature of from 20°–25° C. Carpophores were produced more abundantly during the winter and spring months than during the summer. They were more nearly normal in appearance when grown in semi-darkness.

It was found impracticable to grow *Agaricus campestris* in sufficient quantity *in vitro*. My cultures were grown in a mushroom cellar in the greenhouses of Columbia University. The space available, however, was relatively small, and for comparison it was found desirable to study the general yield, weight, and size of the mushroom as grown in commercial mushroom houses in the vicinity of New York City. The mushroom clusters as they appear in the beds from the very youngest stages were plotted on quadrille paper, and each individual in a cluster was numbered and definitely located so that its whole history could be followed. The horizontal diameter of the apical region of the button was measured from day to day until it reached maturity, *i.e.*, until it became fully expanded or failed to increase in size, turned brown, and died. In this manner the growth curve and fate of each plant were carefully studied. The percentage of buttons that developed normally, the relative rate of growth, and the size and weight of the plant were determined and recorded. The data thus obtained soon enabled me to predict after two days in the case of any particular button whether or not it would reach maturity. The results of these studies I shall report elsewhere; here I wish to report on the gill development as shown in the case of these buttons of known age and normal development.

Fixations were made of white buttons that had just made their appearance on the surfaces of the beds, and of all later stages of normal buttons. Plants also that failed to show any increase in size and those that turned brown were fixed in a great variety of fixing solutions at different intervals. Agar-grown specimens of *Coprinus ephemerus* and *C. stercorarius* were fixed in a great number of different fixing solutions, the best of which was a dilute Flemming's weak solution. The plants were imbedded in paraffin and sections 5 to 25μ were made and stained. Sections of fixed buttons of *Agaricus campestris* were also compared with those of living material.

AGARICUS CAMPESTRIS

The accepted facts as I have described them for *Coprinus micaceus* relative to the development of the simplest carpophore of the so-called endogenous types are: First, that the young, undifferentiated carpophore becomes divided into pileus and stipe rudiments. Second, the primordia of the hymenium arise as a circle or whorl of palisade pockets at or near the lower surface of the pilear rudiment, so that in a longitudinal median section of the young fruit body portions of this series appear as two densely staining narrow areas of hyphae on both sides of the button slightly above the center. Third, the growth of the hyphae of the pileus rudiment is marginal, that is, the youngest portion of the pileus is at its margin. I have pointed out for *C. micaceus* that there is no general annular gill cavity as maintained by Hoffmann, Atkinson, and others for a number of agarics, into which radially arranged plates of hyphae, primordia of the lamellae, grow, but that the palisade cells orient themselves on both sides of vertically arranged interlamellar spaces so as to form a series of gill cavities and gill

Anlagen. Where the ends of the palisade hyphae meet, the interlamellar space, the gill cavity *Anlage*, is at first tightly closed and does not appear as an open interhyphal space even with high magnification. The hyphae between two such adjacently formed palisade layers constitute the *Anlage* of the trama. These hyphae can be traced back into the rudiment of the pileus and into that of the stipe.

The results I obtained in *Agaricus campestris* are similar to those obtained with *Coprinus micaceus.* Atkinson and his students have noted that the fixation of larger types of gill fungi is exceedingly difficult. The large continuous annular gill cavity which they describe is, as I find, an artefact due to poor fixation. I have studied the development of *Agaricus campestris* from material grown under observation in mushroom beds and have been able to follow the development, growth rate, etc., of these plants from the time they appeared on the surface of the bed to the time they are fully expanded. I have compared sections of fresh and fixed material at all stages. It is at once discovered in such studies that not all the buttons which appear in young clusters are destined to reach maturity, as noted by Duggar (1915) and others. Studies of growth rate, etc., enabled me to predict which buttons are going to develop and which are not. Generally, after 72 to 96 hours after the appearance of the buttons on the substratum those that are destined to develop can be distinguished from those that are not. At this time there is no conspicuous difference in their appearance, but only in their ability to grow as shown by their previous rate of growth. Later on, those that do not develop turn brown.

Young plants that showed constant increases in size by actual measurements were sectioned free-hand and studied quickly thereafter under the microscope. The sections showed, in the young and late stages of the development of the palisade pockets and gill primordia, no annular gill cavity. The hymenophore primordia and the rudimentary gills shown in Plate XXVIII, figures 1–16, are firmly attached to the fundamental tissue of the stipe below, just as they are in *Coprinus micaceus*, *C. ephemerus*, and *C. stercorarius* mentioned below. On the other hand, specimens similar as to age and size fixed in the various commonly used fixing solutions showed within twenty minutes after fixation two large holes placed right and left of the stipe (figs. 35–51), just below the young pileus and corresponding exactly to the annular gill cavity described by Atkinson. It should be added that great care must be taken in making these sections since the forming palisade pockets weaken in the tissue connection between pileus and stipe. Young buttons that showed no sign of developing normally and those that turned brown were also studied in free-hand sections. These showed conditions similar to that in the unfixed living plants. There was no annular gill cavity, and the gill *Anlagen* were in connection with the fundamental tissue below the pilear region (figs. 17–22). When the apparently dead carpophores were fixed in chrom-acetic and various other fixing agents they showed no annular gill cavities and did not differ from the unfixed living or unfixed dead specimens (figs. 23–28).

It appears that the already inert cells are less likely to shrink in fixation than are those of the living and growing plants. For this reason fixed material of dead plants was studied, the living plants shrinking too much to give a true picture of the conditions even with the fixing agent generally conceded to be the best for fungi. Figures 29–51 represent free-hand sections of living buttons approximately of the size and age in which gill primordia are developing after having been fixed for 24 hours in the following fixing agents: chrom-acetic, Bouin's picro-formol, picro-acetic Carnoy's, Gilson's, Kaiser's, Flemming's strong, medium, and weak, Juel's, and Merkel's. It appears from these figures that the chrom-acetic, picric acid, and Flemming's strong solutions produce the greatest amount of tearing of the fundamental tissue just below the lamellar region.

Flemming's weak solution produced the least shrinkage of all fixing agents (figs. 29–34), but still sufficient shrinkage to vitiate the results. For this reason it was found that detailed histological studies were best made from fixed dead carpophores simultaneously with a study of free-hand sections of fresh ones. The dead carpophores after being fixed were carried through the alcohols to paraffin with the greatest care and then sectioned.

As to the early development of Agaricus species my results from the fresh and fixed dead plants confirm those of De Bary (1887), Hoffmann (1860), and others. The development of the gill primordia is essentially similar to that in the Coprinus species I have described. The gill cavities originate separately and not as a continuous annular or ring-shaped opening. They arise as arched series of palisade cells which appear very early between the vertically arranged radial plates, the trama primordia, so as to form a series of wide archways, the rudimentary gill cavities. The tramal elements are in connection with the fundamental tissue of the stipe region below. In *Agaricus campestris* the rudimentary gills differ in shape from those of the Coprinus species I have studied, in that they are broader near the pileus and more or less wedge-shaped as they approach the fundamental tissue of the stipe region; so that, when shrinkage of the tissue occurs, one studying a tangential longitudinal section is given the impression that the gills grow downward into a large cavity.

Sections of fresh buttons together with prepared dead carpophores of approximately the same age and size show that the wedge-shaped gill primordia are attached to the fundamental tissue of the stipe region, and very often the gill cavity *Anlagen* are invaded by fundamental tissue from below giving a picture similar to those described by De Bary (1866), Brefeld (1877), and Atkinson (1914*c*) for Amanita and Amanitopsis species. These stages were reproduced satisfactorily only by photographic methods from carpophores that were dead and which were put into Flemming's weak solution, sectioned, and stained, although similar figures were obtained from free-hand sections of the living carpophores.

Figure 52, Plate XXIX, represents a longitudinal tangential section of a young button in which a number of gill *Anlagen* are shown. The margins of these young gills are in connection with the tissue below them, and in the center or oldest part of the section gill cavities are already formed. While this is a microphotograph of a plant that died very early in its development, similar stages were observed in the living buttons. In the unstained sections of young living buttons the gill chambers are wider near the stipe region. The palisade cells which cover the upper surface of the gill chambers are small cells which are not markedly oriented as we find them in the Coprinus species. The trama primordium is very difficult to distinguish in unstained material, yet it has been made out clearly to consist of a narrow band of tissue which is composed of cylindrical cells, which are continuous with the cells in the stipe below and in the pileus above. In the preparation of the dead plant as shown in the above-mentioned figure, the palisade cells do not stain strongly, but a thin, pale violet stain is present between the compact mass of cells of which it is composed. The trama walls stain heavily. The fundamental tissue below the gill cavities consists of a loose plectenchyma which occasionally shows a disintegrating nucleus.

In an older stage of a button, also slightly larger, shown in the fresh carpophores, the tramal elements are in close connection and continuous with the hyphae of the fundamental tissue of the stipe and pileus. The gill primordia are considerably larger, while the palisade cells are longer and have become more numerous but maintain the same shape. In a fixed button that had turned brown at the same age and size, shown in figure 53, the tramal elements are seen clearly in connection with the stipe and pilear tissue. The triangular arch of the gill cavity is covered by palisade cells which stain faintly. The highly granular appearance of the fundamental tissue below the gill rudiment is due to crystal deposits in the cells.

A median and tangential section of another dead carpophore (fig. 55), of the same size but fixed two days after the one shown in figure 53, shows that the palisade cells have largely disappeared and can only occasionally be recognized around the broad and deeply staining tramal tissue. The hyphae of the fundamental tissue are deeply stained also, and, as in the living carpophores of this age, long strands of hyphae can be traced directly into the trama of the young gill rudiments. In the tangential section shown in figure 54 a number of the gills to the right are disconnected from the fundamental tissue below them. This is due to the splitting and collapse of the fundamental tissue as indicated by the more compact plectenchyma of hyphae shown at *B*. This figure is comparable with the figures of Atkinson (1906, figs. 12, 19, 32–38) and Sawyer (1917*b*, fig. 37); these authors, however, failed to recognize that the material they were studying was dead when fixed as evidenced by the disappearance of the palisade cells and the apparent continuity of the tissue of the rudimentary gills with the fundamental tissues after fixing in picro-acetic or chrom-acetic acid, as my experiments show. They explain this structure by assuming that this continuity of tissue is merely contact of the young gill with the rudimentary stipe due to the force exerted on the fundamental tissue by the involute margin of the pileus, bringing this tissue in close contact with the young gill.

Buttons that showed no increase in size and that had been on the surface of the substratum for 48 to 72 hours after they failed to show signs of growth, and fresh buttons of approximately the same age, showed stages in which the young gills were well formed. In those specimens that were already beginning to turn brown, the stretching and expansions of the tissues were retarded. These plants show but slight evidence of an involute margin (Pl. XXX, figs. 59, 60, 61), yet the fundamental tissue of the stipe region is continuous with the gills. In the sections shown in figures 59 and 61, the sagging of the tissue below the gills causes the formation of the deeply stained line which in a photograph tends to give one the impression of an artefact, but under the microscope hyphae can be traced from the fundamental tissue into the young gills. This sagging or collapse of the tissue is associated with loss of turgescence and necrosis. In all three cases the palisade cells are devoid of cytoplasm, and in figures 60 and 61 they seem to have broken down, giving a ragged appearance to the layer, which is generally even and smooth at this stage.

In a study of buttons of *Agaricus campestris* that have turned brown after they had been on the surface of the substratum for 96 hours without showing any signs of growth, we find conditions such as are represented in figures 56, 57, Plate XXIX, and figure 58, Plate XXX. The first of the series (fig. 56) represents a longitudinal tangential section toward the margin. This plant, as we can judge from the median section (fig. 58), has no marked involute margin and is similar to normal buttons of this size and age before death. There is no incurved margin, yet we see deeply stained tramal elements attached firmly to the fundamental tissue (fig. 56). Faint indications of the palisade cells bounding the gill cavities can be seen in this figure. Though these buttons are dead, these sections offer further evidence of the continuity of the gill elements with the pileus region above and with the fundamental tissue of the stipe below. In this preparation and in stages of living plants of the same age we find quite conclusive evidence that the palisade layer is not followed by a region toward the periphery of the hymenophore primordium which subtends an annular gill cavity, as maintained by Atkinson and his students, but that this region is closely in contact with the fundamental tissue below it, as shown here. As our sections approach the median we find that the gills have already become separated from the fundamental tissue, which evidently develops into the annulus. It is of interest to note in these sections near the stipe the furrows of the fundamental tissue which are well shown in figures 57 and 58 directly below the margins of the young gills. Figure 57 represents a view near the stipe region, and we see the gills already torn away from the tissue below. This fundamental tissue below the gills has collapsed partly and has formed a thin, regular curve (in outline), deeply stained, which marks the upper surface of the annulus (*A*). The fine radial grooves which are noticed on the rings of annulate species and on the upper portions of the stipes of exannulate species are marks of this continuity which exists between the hyphae of the stipe and the tramal elements of the gills. Harper's (1913, 1914) excellent photographs of annulate and exannulate fungi bring out these facts clearly. The gills and the furrows seen in outline are approximately equal in number, and the shape of the margin of the gill seems to indicate that it is the counterpart of the furrow of the annulus.

It appears, then, from the study of both the living and the dead buttons of *Agaricus campestris* that the method of the development of the lamellae is essentially the same as in Coprinus species. The early stages in the formation of the gills in Agaricus show more clearly the relation of the tramal elements to the palisade cells and that they form bridges of hyphae between the pileus and stipe regions. The orientation of the palisade cells on both sides of the trama forms typical arches enclosing gill cavities. One apparent difference between the early gill formation in Coprinus and in Agaricus species is that in the latter the palisade cells which arise from the lower portion of the trama are at first shorter than those that arise higher up from the pilear region, thus giving the young gill a wedge shape in section, the gill cavity forming from the beginning a true arch. It becomes clear that, as seen in section, the arch of palisade cells is the unit of structure of the hymenial surface. This palisade tissue originates as a mere pocket which elongates radially with the marginal growth of the pileus. These palisade groups widen on both margins by the formation of new elements which bud out from the trama. These new elements face each other, and the interpalisade space is the forming gill cavity.

Among the normal plants of *Agaricus campestris* grown from pure spawn there often appeared a button which was at first indistinguishable from the other buttons, but which became characterized by the smallness of the pilear region. These plants never mature as far as I have observed. Sections of these buttons were made, and it was found that the basal portion of the plant consisted of an undifferentiated mass of hyphae the elements of which are very narrow and very much intertwined, taking in general a uniform stain. The apical portion, which was marked off by a superficial annular furrow, was found to consist of five zones of tissue (figs. 62–65, Pl. XXXI). The central part consists of a hemispherical mass of intertwining hyphae, recalling at once Fayod's *couche piléogène*. This structure stained heavily. The hyphae in the middle of the central region are very much entangled, as mentioned above. Toward the surface, the hyphae become radiate and blended with a zone of much more faintly staining hyphae immediately above. This in turn is followed by another broad, densely staining area, and this is succeeded by two other layers also staining deeply but not so wide. The significance of these regions is not clear, but it appears that by differentiation of the tissue between the *couche piléogène* and the light-staining tissue above it, the reproductive tissue is formed. Figures 64 and 65 show a median longitudinal section of the pilear region in which differentiation has set in and a number of poroid gills have been formed. The tramal elements shown in the enlargement (fig. 65) take the stain heavily, but the palisade cells are too faint to register an impression on the photographic plate. Such appearances as this suggest the possible origin of the gilled fungi from a poroid type.

Coprinus ephemerus and C. stercorarius

The young carpophores of *Coprinus ephemerus* and *C. stercorarius* generally appear at the margin of the agar culture and seem to arise from a comparatively stout rhizomorph-like strand of hyphae. Very often a small piece of agar containing hyphae of *C. ephemerus* and *C. stercorarius* transferred to another culture medium produced small greyish sclerotia, as shown by Brefeld (1877). Mycelial growth then continues, radiating from the inoculum to the margin of the agar where the carpophores are formed. Not uncommonly, small carpophores arranged in a radial series arise from a rhizomorph close to the sclerotium, as shown in figure 66 (Pl. XXXII). Subsequently minute carpophores arise all over the agar, as shown in figures 67 and 68. Young carpophores were fixed in Flemming's weak solution diluted to one quarter strength. The earliest stages in the development of *C. ephemerus* resemble in the main the stages described for *C. micaceus* (Levine, 1914). The young, undifferentiated button, less than .1 mm. in diameter, consists of a very much entangled mass of hyphae. The carpophore stains uniformly, although the enveloping outer hyphae end in large globose cells having thick walls. These cells very often stain heavily with the gentian violet of the triple stain. The primordium of the pileus does not stain differentially as readily as does that of *C. micaceus*, and consequently it is not easily recognized at first. The primordium of the hymenium, however, takes the stain heavily and is the first structure to be noticed in the differentiation of the carpophore. It consists of a horizontal series of palisade pockets and appears in the upper third of the young button; in longitudinal median section it appears, as it has been often described for other species of agarics, as two densely stained areas to the right and left of a median vertical line. In *C. stercorarius* (fig. 85, Pl. XXXV), the young, undifferentiated carpophore consists of a weft of mycelium in which strands of parallel hyphae can be traced for a considerable distance, the entire mass forming a more or less ovoid body and so differing from the corresponding stage of *C. ephemerus* and *C. micaceus*. In this species, also, one may notice that the terminal cells of the hyphae do not become globular at this stage, but that the tips seem to converge toward the apex of the small button. The tip cells are more densely filled with cytoplasm and take the stain more heavily. It is interesting to note that the carpophore shown in figure 85 arose below the surface of the agar, and it appears in this figure as if a number of hyphae were involved in the initial development of the plant. Sections of small knob-like projections, which give rise to carpophores and which appear on the stipes of older but attenuated specimens of *C. stercorarius*, consist at first of an undifferentiated mass of hyphae, as shown in figure 86. Here it may be observed that, while the cells of the old stipe are large and generally highly vacuolated, the cells from which the young carpophore seems to take its origin are small and stain heavily. This type of young carpophore is somewhat more compact in structure, and the base of the plant stains less strongly than the apical region. In *C. stercorarius*, unlike *C. ephemerus*, the first change in the undifferentiated carpophore consists in the formation of a pilear region which answers the description of Fayod's (1889) *couche piléogène* (fig. 87). It appears to be an inverted flattened hemispherical mass of tissue consisting of very much entangled, deep-staining hyphae. The stipe region at this stage also stains somewhat deeply, and between the two a layer of undifferentiated, delicately stained tissue remains. The apical terminal cells of this plant are thick-walled; they lose their cytoplasm and fail to stain. It is quite likely that it is these cells which form the flaky structures on the surface of the mature pileus later in the development of the plant.

In both *C. ephemerus* and *C. stercorarius*, these early stages are followed more gradually by the development of pockets of palisade-like hyphal tips immediately below the pilear region. In *C. ephemerus* the undifferentiated primordium of the hymenophore region gives rise to these pockets of palisade cells, no distinct pilear region having been formed; in *C. stercorarius* they are formed on the outer part of the lower surface of the pileus rudiment. Previous to this stage, and during the development of subsequent stages, there is no indication of a general annular gill cavity such as is described by Atkinson and his students for other species of agarics. As I have pointed out above, the matter of successful fixation is of prime importance at this stage of development. It appears that the hyphae that are continuous between the stipe fundament and pilear region, the future trama, are very easily ruptured by shrinkage in fixation.

Gill Cavities

The palisade pockets of hyphal cells arise primarily from the pilear region and appear in longitudinal sections in distinct groups with the downward-growing hyphal tips slightly converging toward a common center within the group. The hyphae on the boundaries of such groups, the future trama cells, are continuous, as noted above, between the hyphae of the pilear and stipe rudiments.

Figure 69, Plate XXXII, represents a longitudinal tangential section of a very young carpophore in which there is no annular gill cavity, but the palisaded cells are arranged quite obviously into at least four distinct groups with three faintly stained trama areas (*A*) between them which are continuous with the stipe and pileus rudiments. As we shall see later, where the tips of the oriented palisaded cells of each pocket meet, a gill cavity will be formed. The strictly median longitudinal sections of the young carpophores which lie in the plane of the future gill cavity are, it is obvious, less favorable for the correct understanding of the gill origin than longitudinal tangential sections. Figure 70 represents such a longitudinal median section of the same button shown in figure 69; the group of cells faintly stained at the right is a section of the area of cells which form the boundary between two adjacent clusters of oriented palisaded hyphal cells. The cluster of deeply stained cells to the left represents a section through the palisade cells. Where the tips of the cells meet we have a radial view of the beginning of the gill cavity. It is already clear in this carpophore that the palisaded cells are well established, and yet there is no evidence of an annular gill cavity.

Figure 71, Plate XXXIII, represents a longitudinal median section of a young carpophore a little older than the one previously described. Here, as in figure 70, we find the two characteristic patches of palisade cells to the right and left, slightly above the center of the carpophore, toward the margins of the young pileus. There is still no indication of an annular gill cavity. As our sections become more tangential (fig. 72) we notice the grouping of the palisaded cells (at *A*) and also the more parallel hyphae which lie between the groups of oriented cells and will form the trama. In a section more eccentric shown in figure 73, there are four distinct groups of palisade cells and three pronounced tramal regions between them (at *C*). Within each group of palisaded cells there are already faint indications of gill cavities which cannot merge into one annular gill cavity unless the tramal cell connections are torn. The primordium of the hymenium is oldest nearest the stipe. Toward the margin it becomes progressively younger. Figures 71, 72, and 73 show a point of interest in that the layer of terminal cells seems to form a distinct covering and appears to correspond to what Brefeld called a general veil. This layer has been torn away from the pilear region in the process of sectioning.

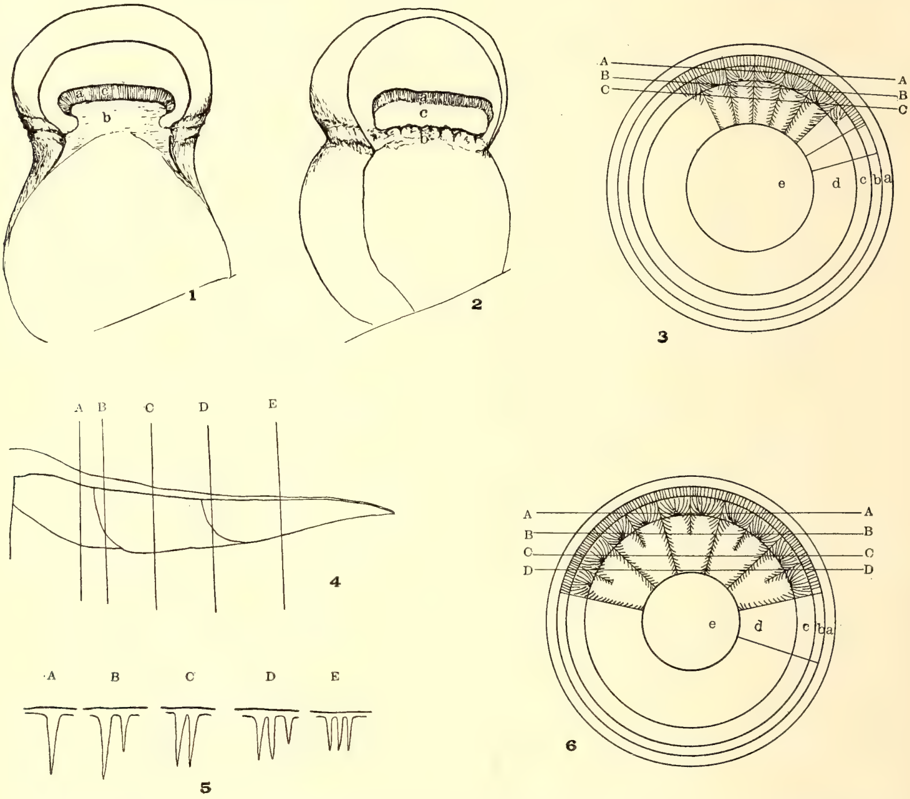
A somewhat older carpophore is shown in figure 74. The section is longitudinal and not quite median, through two groups of palisade cells with their small gill cavities in the earliest stages of appearance. More tangential sections of this carpophore (fig. 75) show four distinct groups of oriented palisaded cells (at *A*) with their rudimentary gill cavities and the intervening tramal cells. A distinct and conspicuous gill cavity is visible in this figure in the second group of palisade cells from the left side. In a section of the same plant still more tangentially placed and shown in figure 76, we have three groups of palisaded hyphal cells and the gill cavities have entirely disappeared; that is, we have reached that portion of the hymenial primordium in which the palisaded cells while oriented have not as yet sufficiently separated to form a cavity. There is, of course, considerable fluctuating variability in the arrangement and the size of the gill chambers, and the sections are by no means exactly parallel to the longitudinal axis of the young carpophore. This axis, again, need not be a perfectly straight line. These facts will account for many apparent inconsistencies in the sections. It is, however, sufficiently clear from the figures that these rudimentary gill cavities arise at the region where the tips of the oriented palisaded cells meet. They become larger by an increase in the number of the palisade hyphae which seem to take their origin in the pileus and the tramal cells. The result is an arched rudimentary hymenial surface, under which the gill cavity becomes larger and larger as this hymenial surface increases and the walls of the two adjacent arches with their common trama become a single gill. In perspective we should have a series of these radially arranged arches and tramal plates, older and larger near the stipe and younger and smaller toward the margin. The tramal plates remain firmly attached to the stipe tissue below since their hyphae run through from pileus to stipe. The growth of the pileus rudiment is centrifugal, so that, while the rudimentary arch or gill cavity may be a wide opening near the stipe, it is just unfolding or opening at the margin of the pileus.

Tangential sections of older buttons are represented by figures 77, 78, and 79. In the middle of such a section the gill chambers are distinct openings (figs. 78, 79), taking the shape of Roman arches; toward the right and left they dwindle away, and we have only a pocket of hyphae with their tips pointing to a common center and no visible opening (fig. 77), In the last mentioned figure we find the two gill cavities still unopened or in a very rudimentary state. Each palisade cell contains two nuclei which stain well with Flemming's triple stain. There is no cytological indication at this stage that the older cells are to be found near the bases of the arches or rudimentary lamellae, as Buller (1909) suggested for the mature gills, and yet this observation does not indicate an inconsistency, for it is quite possible that the hymenium may mature and develop later from the margin of the pileus and outer edge of the gill upward, and be independent of the time of origin.

As we proceed still farther toward the center of the plant a large number of gill cavities appear, as shown in figure 79, which represents the maximum number that appear in a longitudinal tangential section of this button.

Figure 77 becomes more intelligible when studied in connection with diagram 3, which represents a top view of the carpophore in the earliest stages of development. The outermost space (*a*) between the two outer circles represents the region of the fundamental and undifferentiated tissue.

The next space toward the center (*b*) with small radial lines represents the smooth, unoriented palisaded layer of hyphal cells which is followed by a region (*c*) of oriented palisaded cells, and this in turn by the older portion



Diagrams 1–6. 1. Longitudinal tangential section of a fresh carpophore showing the young lamellae (*a*), attached to the fundamental tissue (*b*), and forming a number of interlamellar spaces (*c*). 2. Longitudinal tangential section of a fresh carpophore after having been fixed from 6 to 24 hours. The young lamellae (*a*) are torn away from the fundamental tissue (*b*), leaving a wide annular gill cavity (*c*). 3. Top view of a carpophore in the earliest stages of development; the outermost space (*a*) represents fundamental and undifferentiated tissue; (*b*) smooth, unoriented, palisaded layer of hyphal cells; (*c*) oriented palisaded cells; (*d*) older portion of hymenophore primordium with gill cavities and gills; (*e*) stipe rudiment. 4. Radial section of pileus showing three gills of different lengths. 5. Various widths of gills shown by vertical cross sections made at various distances, *A*, *B*, *C*, etc., from the stipe. 6. Top view of carpophore showing secondary gills.

of the hymenophore primordium (*d*), that is, where distinct gill cavities and gills are already formed. The inner space represents a portion of the stipe rudiment (*e*). The line *AA* represents a vertical tangential section shown in figure 77, cutting through and exposing two groups of palisaded cells at the tips of which we find the youngest stage of two gill cavities. The line

BB represents the section shown in figure 78, where the two *Anlagen* of the gill cavities shown in figure 77 have opened and the two halves of the adjacent verticals of the arch form the gill; at the same time on both sides of these two gill cavities appear the eccentric views of two more gill cavities which are shown in figure 79, the approximate location of the section being represented in the diagram by the line *CC*.

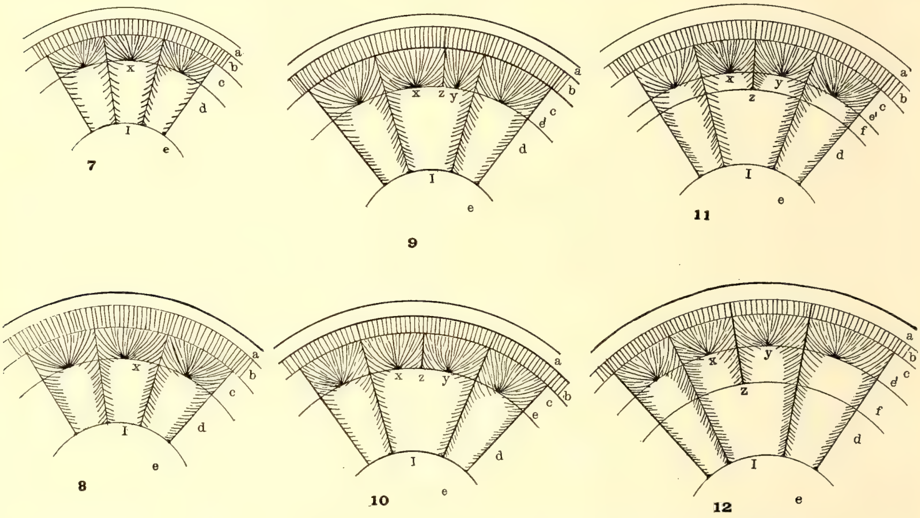
Secondary Gills

In buttons slightly older than those previously described we can study the origin of secondary gills. The origin of these gills is of special interest since the inner end of the gill often appears in a single longitudinal section (Douglas, 1916, fig. 50) as a narrow gill apparently growing downward into the gill cavity. The secondary gills are merely short gills extending from the margin of the pileus part way to the stipe.

In diagram 4 we have a primary gill and two shorter ones superimposed. The line *A* cuts through the primary gill which extends from the stipe to the margin of the pileus, and its width is represented in diagram 5, *A*. Here we have the full width of the gill. The line *B*, diagram 4, cuts through the primary and one of the secondary gills, and the widths of the two gills are shown in diagram 5, *B*. The secondary gill through the region *B* is narrower than we find it at *C*. Here the primary and the secondary gill are of the same width. Similarly, we find the shortest gill narrower than the two preceding gills through *D*, but it becomes of equal width in the plane *E*.

Coprinus ephemerus is especially favorable material because of the small number of gills and the relatively late appearance of the secondary gills. Their development is entirely similar to that I have already described for *Coprinus micaceus*. In the tangential section shown in figure 81 we are near the margin, as shown in diagram 6 by the line *BB*. In this section we find a narrow gill. The second gill cavity from the left shown in figure 82 represents the left-end one in figure 81, and the third gill cavity represents the larger middle one in which the secondary or short gill appears to hang freely in figure 81. This short gill is not actually free through all its length as it would be if it were developing and growing down into the gill chamber, for in the succeeding parallel tangential section shown in figure 80, made toward the outer surface of the carpophore at a region indicated by the line *AA* in diagram 6, we have the four *Anlagen* of the gill cavities and the younger or marginal part of the secondary gill firmly joined to the fundamental tissue below. Notice how closely the two middle gill-cavity *Anlagen* lie to one another. The crowded condition is indicative of the development of a gill cavity in a space which formerly allowed only one. The tramal region recognizable between the *Anlagen* of the other gill cavities is much greater and wider, showing an undisturbed condition since no new cavities are forming.

It appears, then, that as the circumference of the young carpophore becomes greater the distance between the young lamellae at the margin becomes greater, and in the regions of the fundamental tissue and of the palisade cells between these primordia of the lamellae, further differentiation of these tissues occurs which results in the formation of a new tramal plate bounded by palisade cells, which thus form a new gill—the short or secondary gill. The old gill cavity is split by the new gill. The apparent suspension of the secondary gill is due to the growth of the inner margin of this gill toward the stipe (see diagram 4), giving this edge a curved outline. Diagrams 7 to 12 inclusive represent the development of a secondary gill. The letter *a* represents the undifferentiated region of the pilear structure, *b* the smooth palisade cells, *c* the oriented palisaded cells, *d* the young gills, and *e* the stipe region; *e′* represents a region where the undifferentiated fundamental tissue of the stipe and pilear regions, at the periphery of the carpophore, meet; *f* represents the increase in growth of the secondary gill.



Diagrams 7–12. Top view of a series of carpophores showing the development of a secondary gill at "*Z*" by the development of a secondary gill cavity at "*Y*." *e′* represents a region where the undifferentiated fundamental tissues of the stipe and pilear regions meet at the periphery of the carpophore; *f*, the increase in growth of the secondary gill.

This series of diagrams shows a carpophore increasing gradually in size. With growth, the distance between the lamellae at *I*, diagram 7, becomes greater, and more palisaded cells appear between the tramal elements as shown at *x* in diagram 8. A new center upon which the new palisade cells become oriented, at *y* in diagram 9, and a point *z* on the stipe fundament *e′*, become established with the differentiation of a new rudimentary trama, the hyphae of which are continuous between the stipe and the pilear regions. Continued growth upward into the pileus (diagram 10) makes the new center *y* as fully developed as *x*, and subsequent development brings about the opening of the palisade cells *x* and *y*, forming a young secondary gill which divides the old gill cavity into two. The young gill is fixed at *z* because of the continuity of the tramal elements between the pileus and stipe hyphae in this region. Further development of the gill cavities increases the width of the primary and secondary gills represented by *f* in diagrams 11 and 12. Growth takes place peripherally to *z*, at *x* and *y*.

Very often these secondary gills break away from the fundamental tissue below them so that they remain somewhat narrower than the primary gills. It appears from these sections and from hundreds of similar ones that the secondary gills have a development identical with that of the primary gills except that their point of origin is at some distance from that of the primary gills.

The cells of the stipe resemble those of other species of Coprinus in that they are multinucleated. The globular cells on the surface of this button shown in figure 83, Plate XXXIV, disappear at a later stage, as shown in figure 84. The cells making up the trama also disappear up to and through the pileus, so that the hymenial surface alone appears in older buttons. This condition gives the pilear surface its fluted or corrugated appearance. Figures 91, 92, and 93 represent cross, longitudinal tangential, and longitudinal median sections of the older carpophores of *Coprinus stercorarius*. The cells covering the pileus of this species apparently plasmolyze readily and give the appearance of globular cells with an irregular dark-staining body in their interior. These are intermingled with cylindrical hyphal threads which also appear to be plasmolyzed. These cells give the scaly appearance of the mature pileus. I have observed carpophores with attenuated stems like those described by Brefeld. The pilei are much reduced and the stipes are very long, as shown in figure 88, Plate XXXV. The gills are merely narrow ridges as shown in figures 89 and 90, the latter of which represents a longitudinal tangential section. These forms are plainly abnormal. They may be found in material collected in the field among the belated specimens.

I have also studied sections of *Coprinus atramentarius* made from material collected in the field. Buttons in all stages were found in a cluster that appeared close to a group of plants already mature. These young plants were fixed in diluted Flemming's weak solution, and the stages studied agree with those described for *C. micaceus*. No annular gill cavities were observed. Figures 94 and 95 represent median and tangential longitudinal sections respectively. The young gill *Anlagen* in both cases are attached to the fundamental tissue of the stipe region. It is clear from the data given above that, in the species of Coprinus so far described, the development of the carpophore and the formation of the gills are practically the same, with but slight variations as to the time of formation of the stipe or of the pileus.

Summary

1. Fresh buttons of *Agaricus campestris* fixed in the standard fixing agents show shrinkage and tearing of the fundamental tissue within 6 to 24 hours after fixation.

2. Fresh and dead carpophores of *A. campestris* studied show no primary annular gill cavity but a series of arches or gill cavities between each pair of gills with the trama tissue continuous with the hyphae of the pileus and of the stipe rudiments. There is no one general annular gill cavity but a number of interlamellar cavities similar to those found in the Coprinus species.

3. Spores of *Coprinus ephemerus* and *C. stercorarius* sown on dung or bean agar produce carpophores within 10 days after the inoculation is made.

4. The early stages in the development of the carpophore *Anlage* in these species is similar to that in *C. micaceus*. The early stages in the differentiation of the carpophore of these species are similar, up to the time of the development of the lamellae, to those of other types of agarics already described. The development of the lamellae of *C. ephemerus* and *C. stercorarius* is similar to that described by me for *C. micaceus*. No general annular gill cavity is formed.

5. The primordium of the hymenium arises as pockets of palisade cells with the ends of these cells pointing downward. As they increase in number they form a small arch, enclosing thus an interhyphal space between their free ends. This interhyphal space is the beginning of a gill cavity. The palisade cells forming the vertical walls of the arch are the rudiments of the adjacent hymenia of two gills. The vertical plate of hyphae between two adjacent arches constitutes the rudimentary trama, which is continuous with the hyphae of the stipe below and of the pilear fundament above. The young gill in its earliest stages of development is composed of the tramal cells together with the adjacent vertical walls of two arches.

6. Secondary or short gills arise in a manner similar to that of the primary gills. As the pilear rudiment increases in diameter, the distance between two adjacent rudiments of primary lamellae of the margin becomes greater and greater. A new pocket of palisade cells is intercalated at the margin of the pileus between two pockets of cells already formed and which are already open near the stipe. A cluster of new tramal hyphae is also formed which is fixed and continuous between the stipe and the pileus, as are the trama cells of the primary gills. As the palisade cells of the new pocket and the cells of the already formed adjacent pocket increase, two small arches are formed, separated by the newly formed trama and the adjacent hymenia. The trama and the adjacent hymenial surfaces constitute the short gill. This divides the former gill cavity into two.

7. The short gills are attached to the stipe fundament from the very beginning and do not grow downward between two old gills.

Columbia University

LITERATURE CITED

Adams, J. F. 1918. Origin and development of the lamellae in *Schizophyllum commune.* Mem. Torrey Bot. Club **17**: 326–333. Pl. 9, figs. A, B.

Atkinson, G. F. 1906. The development of *Agaricus campestris.* Bot. Gaz. **42**: 241–264. Pls. 7–12.

——. 1914*a*. The development of *Agaricus arvensis* and *A. comtulus.* Amer. Jour. Bot. **1**: 3–22. Pls. 1, 2.

——. 1914*b*. The development of *Lepiota clypeolaria.* Ann. Myc. **12**: 346–356. Pls. 13–16.

——. 1914*c*. The development of *Amanitopsis vaginata.* Ann. Myc. **12**: 369–392. Pls. 17–19.

——. 1914*d*. The development of *Armillaria mellea.* Myc. Centralbl. **4**: 113–121. Pls. 1, 2.

——. 1915. Morphology and development of *Agaricus Rodmani.* Proc. Amer. Philos. Soc. **54**: 309–343. Pls. 7–13, figs. 1–8.

——. 1916*a*. Origin and development of the lamellae in Coprinus. Bot. Gaz. **61**: 89–130. Pls. 5–12, figs. 1–6.

——. 1916*b*. The development of *Lepiota cristata* and *L. seminuda.* Mem. N. Y. Bot. Gard. **6**: 209–228. Pls. 21–26.

Bonorden, H. F. 1851. Handbuch der allgemeinen Mycologie. Stuttgart.

Brefeld, O. 1877. Botanische Untersuchungen über Schimmelpilze. Heft 3. Basidiomyceten **1**: 1–135. Pls. 1–9.

Buller, A. H. R. 1909. Researches on fungi. London.

De Bary, A. 1866. Morphologie und Physiologie der Pilze, Flechten und Myxomyceten. Leipzig.

——. 1887. Comparative morphology and biology of the fungi, mycetozoa, and bacteria. (Eng. transl.) Oxford.

Douglas, Gertrude E. 1916. A study of development in the genus Cortinarius. Amer. Jour. Bot. **3**: 319–335. Pls. 8–13.

Duggar, B. M. 1915. Mushroom growing. New York.

Dutrochet, H. J. 1837. Mémoires pour servir á l'histoire anatomique et physiologique des végétaux et des animaux **2** : 370–377. Pl. 22.

Fayod, V. 1889. Prodrome d'une histoire naturelle des Agaricinés. Ann. Sci. Nat. Bot. VII, **9**: 181–411. Pls. 6, 7.

Harper, E. T. 1913. Species of Pholiota and Stropharia in the region of the Great Lakes. Trans. Wis. Acad. Sci., Arts, Lett. 17^2: 1011–1026. Pls. 59–67.

——. 1914. Species of Hypholoma in the region of the Great Lakes. Trans. Wis. Acad. Sci., Arts., Lett. 17^2: 1142–1164. Pls. 72–84.

Hartig, R. 1874. Wichtige Krankheiten der Waldbäume. Berlin.

Hoffmann, H. 1856. Die Pollinarien und Spermatien von Agaricus. Bot. Zeit. **14**: 137–148, 153–163. Pl. 5.

——. 1860. Beiträge zur Entwickelungsgeschichte und Anatomie der Agaricinen. Bot. Zeit. **18**: 389–396, 397–404. Pls. 13, 14.

——. 1861. Icones analyticae fungorum. Giessen.

Levine, M. 1913. Studies in the cytology of the Hymenomycetes, especially the Boleti. Bull. Torrey Bot. Club **40**: 137–181. Pls. 4–8.

——. 1914. The origin and development of the lamellae in *Coprinus micaceus.* Amer. Jour. Bot. **1**: 343–356. Pls. 39, 40.

Nees von Esenbeck, C. G. 1816. Das System der Pilze und Schwämme. Würzburg.

Sawyer, W. H. 1917*a*. Development of some species of Pholiota. Bot. Gaz. **64**: 206–229. Pls. 16–20.

——. 1917*b*. The development of *Cortinarius pholideus.* Amer. Jour. Bot. **4**: 520–532. Pls. 28, 29.

Schmitz, J. 1842. Mycologische Beobachtungen als Beiträge zur Lebens- und Entwickelungsgeschichte einiger Schwämme aus der Klasse der Gasteromyceten und Hymenomyceten. Linn. **16**: 141–215. Pls. 6, 7.

Trog, J. B. 1837. Ueber das Wachsthum der Schwämme. Flora **20**: 609–618.

Walker, Leva B. 1919. Development of *Pluteus admirabilis* and *Tubaria furfuracea.* Bot. Gaz. **68**: 1–21. Pls. 1–5, figs. 1–8.

DESCRIPTION OF PLATES

Plate XXVIII

Buttons of *Agaricus campestris* vars. *Bohemia* and *Columbia.*

Figs. 1–16. Stages in development of fresh carpophores showing the young gills attached to the fundamental tissue below the pilear region. Figs. 4, 9, 13, 14, and 15 are tangential sections, showing no annular gill cavity and the small gills still attached to the tissue which will develop into the annulus. Photographed 5 to 20 minutes after free-hand sections were made. × $2\frac{1}{2}$.

Figs. 17–22. Young buttons which have turned brown and died 48 to 96 hours after their appearance on the substratum. Longitudinal sections show no annular gill cavities, but the gill *Anlagen* are attached to the fundamental tissue below them. Photographs made from buttons 5 to 20 minutes after being sectioned free-hand. × 3.

Figs. 23–28. Dead buttons of the same stage of development after having been in a chrom-acetic solution for 24 hours show no annular gill cavities; the rifts below the young gills were caused by cutting the fluid-soaked buttons. × 3.

Figs. 29–34. Fresh, actively growing buttons sectioned longitudinally after being exposed to Flemming's weaker solution for 48 hours; the so-called annular gill cavities appear and the young gills appear to be growing downward into them. Photographs were made from 5 to 20 minutes after removal from fluid. × 3.

Figs.35–41. Young, actively growing buttons which were subjected to a chrom-acetic solution for 2 hours show exceedingly large annular gill cavities below the developing lamellae. Figures 35, 38, and 40 are tangential sections showing the so-called annular gill cavity as a wide slit due to the shrinkage of the tissue caused by the fixing agent. × 3.

Figs. 42–44. Actively growing buttons, after being fixed in Flemming's strong solution 6 to 18 hours, show shrinkage which causes the development of the annular gill cavity.

Figs. 45–51. Fresh buttons after having been fixed for from 6 to 18 hours: figure 45, in Gilson's solution; figure 46, in Juel's; figure 47, in Bouin's; figure 48, in Merkel's; figure 49, in Kaiser's; figure 50, in picro-acetic; figure 51, in Carnoy's. All × 3. All show tremendous shrinkage, which accounts for the appearance of the annular gill cavity.

Plate XXIX

The microphotographs on this and the following plates were made with the aid of the Zeiss microphotographic apparatus and Leitz objectives nos. 3 and 6, and oculars nos. 1, 3, and 4.

Figures 52 to 62 represent sections of *Agaricus campestris.*

Fig. 52. Longitudinal tangential section of a young dead carpophore, showing the trama of the young lamellae continuous with the fundamental tissue below the pileus and forming a series of interlamellar chambers. Ocular 1, objective 3, bellows 20 cm.

Fig. 53. A dead button slightly older than the one shown in the previous figure, cut tangentially to the long axis of the carpophore; it shows wide tramal tissue and the hymenia. The trama is continuous with the fundamental tissue below. Ocular 3, objective 4, bellows 20 cm.

Figs. 54, 55. Longitudinal tangential and median sections of a young dead button slightly older than the one shown in figure 53. The hymenial surface is beginning to disintegrate and the fundamental tissue to collapse, as shown in figure 54. Continuity of the hyphae between the trama and the fundamental tissue may still be seen in both figures. Ocular 4, objective 3, bellows 20 cm.

Figs. 56, 57. Sections of a young dead carpophore 96 hours old after it had failed to show signs of further development. Fig. 56. Longitudinal tangential section near the margin of the pilear region, showing the trama as more heavily stained columns of tissue continuous with the less deeply stained hyphae of the fundamental tissue below. Fig. 57. Longitudinal tangential section near the stipe, showing the tearing away of the trama of the young gills from the fundamental tissue, the annulus, resulting in the furrows on its surface at *A*. Ocular 4, objective 3, bellows 20 cm.

Plate XXX

Fig. 58. A longitudinal median section of the same carpophore shown in figures 56 and 57, showing the young gills torn away from the fundamental tissue. Ocular 4, objective 3, bellows 20 cm.

Figs. 59–61. Longitudinal median sections of young carpophores which had turned brown after being on the bed 48 to 72 hours and having failed to show signs of growth, showing the trama of the young gills continuous with the fundamental tissue; the involute margin is not strongly developed. Ocular 4 (figure 61, ocular 1), objective 3, bellows 20 cm.

Plate XXXI

Fig. 62. Longitudinal median section of abnormal button showing a densely staining, bowl-shaped central structure comparable to Fayod's *couche piléogène*. Ocular 4, Spencer objective 32 mm., bellows 20 cm.

Fig. 63. Enlargement of part of figure 62, showing the structure of the *couche piléogène*. Ocular 1, objective 3, bellows 20 cm.

Fig. 64. Longitudinal median section of abnormal button slightly older, showing poroid gills to the right and left of the *couche piléogène*. Ocular 4, Spencer objective 32 mm., bellows 20 cm.

Fig. 65. Enlargement of figure 64, showing more clearly the nature of the poroid gills. Ocular 3, objective 3, bellows 20 cm.

Plate XXXII

Figures 66 to 84 inclusive represent *Coprinus ephemerus*.

Fig. 66. A pure culture grown in Petri dishes on dung agar, showing sclerotia and radiating rhizomorphs on which minute carpophores are beginning to appear.

Fig. 67. A later stage, with much branched and anastomosing mycelium covered with great numbers of young carpophores.

Fig. 68. A pure culture, showing an abundance of young carpophores near the margin of the dish; in the center, a mass of sclerotia surrounded by a weft of aerial hyphae.

Figs. 69, 70. Microphotographs of a young carpophore. Fig. 69. Longitudinal tangential section, showing four pockets of palisade cells with three groups of intervening tramal tissue continuous between the pilear and stipe regions shown at *A*. Fig. 70. Median longitudinal section of the same carpophore; the cluster of deeply staining cells to the left represents a radial view through a pocket of palisade cells. The less deeply stained cluster of cells is a section through the trama. Ocular 3, objective 6, bellows 40 cm.

Plate XXXIII

Figs. 71–73. A series of sections of a young carpophore grown on agar. Fig. 71. Longitudinal median section of a young carpophore through two clusters of oppositely placed pockets of palisade cells. Fig. 72. Longitudinal tangential section showing three clusters of oriented palisade cells at *A*. Fig. 73. Tangential section showing four clusters of palisade cells and three intervening groups of tramal hyphae at *C*. Ocular 3, objective 6, bellows 40 cm.

Figs. 74–76. A series of sections of a carpophore slightly older than the one shown in previous sections. Fig. 74. Longitudinal median section through two oppositely placed pockets of palisade cells. Fig. 75. Longitudinal tangential section showing four pockets indicated by *A*, in which the second pocket from the left is beginning to open, forming an interlamellar space. Fig. 76. Longitudinal tangential section through the margin of the carpophore, showing three pockets of palisade cells (*A*) not yet open. Ocular 3, objective 6, bellows 40 cm.

Figs. 77–79. A series of sections of a carpophore still older. Fig. 77. Longitudinal tangential section near the margin of the pilear region, showing two pockets of palisade hyphae. Fig. 78. Longitudinal tangential section near the stipe, showing two well-developed interlamellar cavities; two pockets of palisade cells on either side at the margin. Fig. 79. Longitudinal tangential section still nearer the stipe, showing four distinct interlamellar cavities and a marginal pocket of palisade cells to the left. Ocular 3, objective 6, bellows 40 cm.

Figs. 80–82. A series of sections of a young carpophore with secondary gills. Fig. 80. Longitudinal section near the margin, showing four pockets of palisade cells; the middle two lie close together, indicating the presence of a secondary gill between them, as shown in figure 81 which is a longitudinal tangential section nearer the stipe showing a short gill which in more eccentric sections is attached to the fundamental tissue below it. Fig. 82. A section still nearer the median, showing the disappearance of the short gill and the presence of four gill cavities and three young lamellae continuous with the fundamental tissue below them. Ocular 4, objective 3, bellows 50 cm.

Plate XXXIV

Fig. 83. Longitudinal section near the stipe, showing attachment of mature gills to the fundamental tissue. Ocular 3, objective 3, bellows 45 cm.

Fig. 84. Cross section of a carpophore showing the disintegration of the fundamental tissue between the gills and stipe; no spores are present at the margins of the gills. The globular cells seen on the surface of the pileus in earlier stages have disappeared, leaving a fluted surface. Ocular 3, objective 6, bellows 20 cm.

Fig. 91. Portion of a cross section of the pileus of *Coprinus stercorarius*, showing the attachment of the primary gills to the stipe.

Fig. 92. Portion of a longitudinal tangential section of a carpophore of the same age, showing the young gills still attached to the fundamental tissue; it shows clearly the type of cells covering the pileus which give it its scaly appearance.

Fig. 93. Portion of a longitudinal median section. Ocular 1, objective 3, bellows 20–25 cm. for figures 91–93.

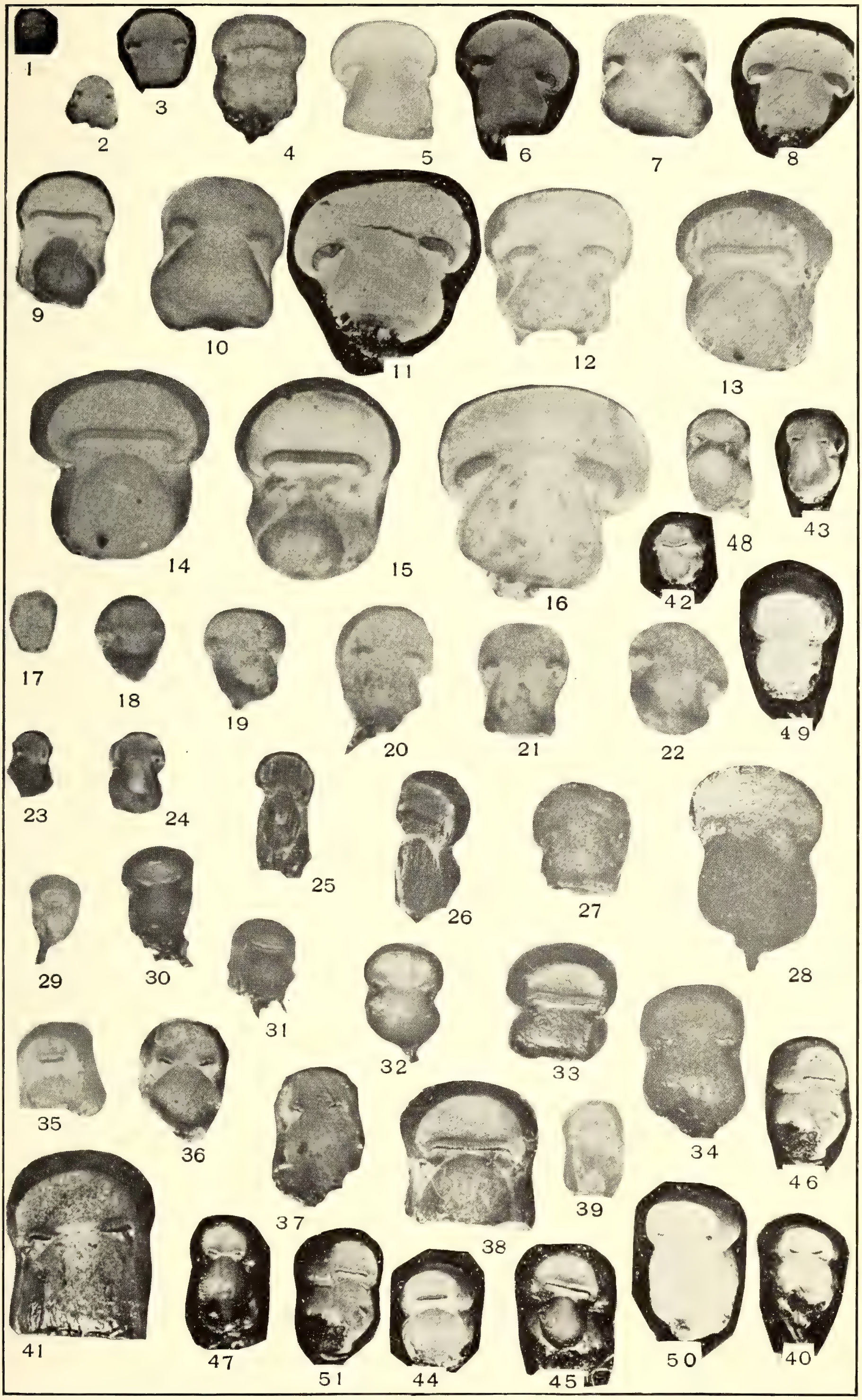
Plate XXXV

Fig. 85. Early stages in the development of a carpophore of *C. stercorarius* grown on agar. The weft of hyphae arising from a number of cells below the surface of the agar, shown at *A*. Ocular 4, objective 3, bellows 20 cm.

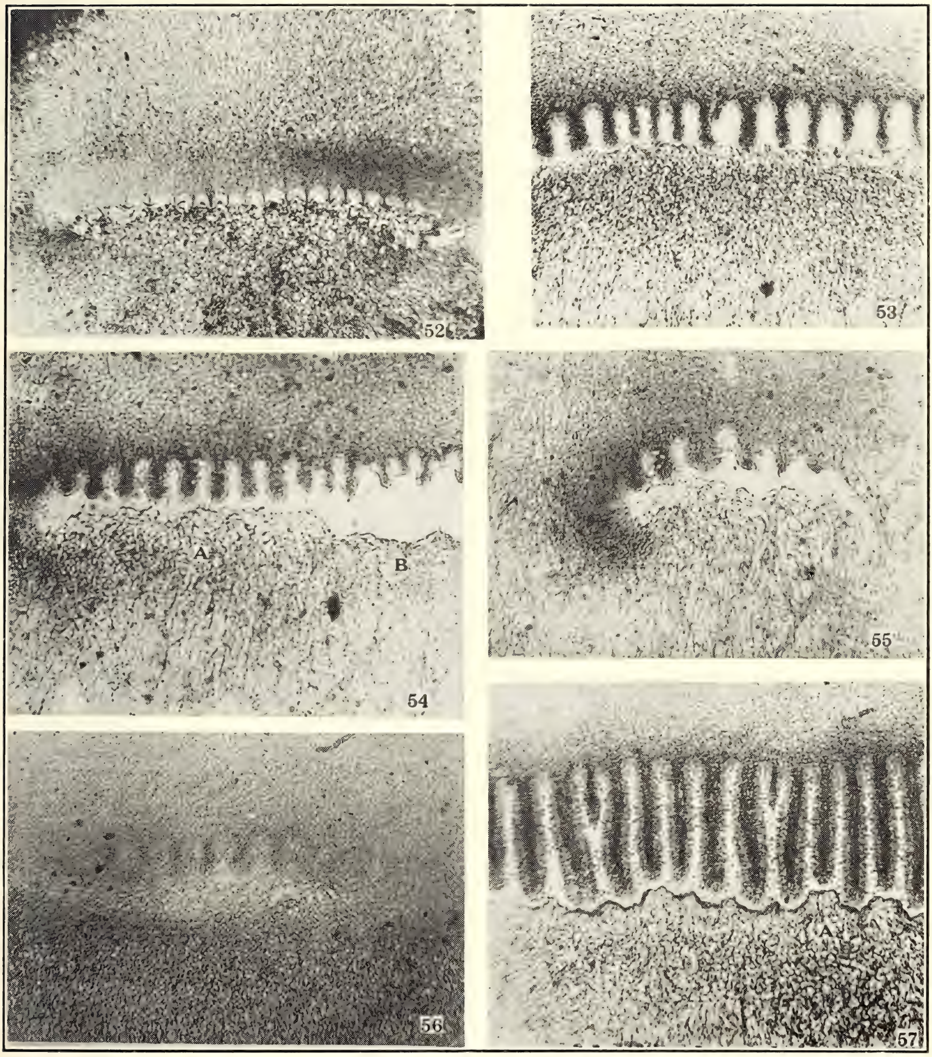
Fig. 86. Early stages in the development of a carpophore of *C. stercorarius* on the stipe of an older button. *B*, a portion of the cross section of the older stipe. Ocular 4, objective 3, bellows 20 cm.

Fig. 87. Early stages in differentiation of a young carpophore of *C. stercorarius*, showing the development of the pilear mass. Ocular 4, objective 3, bellows 20 cm.

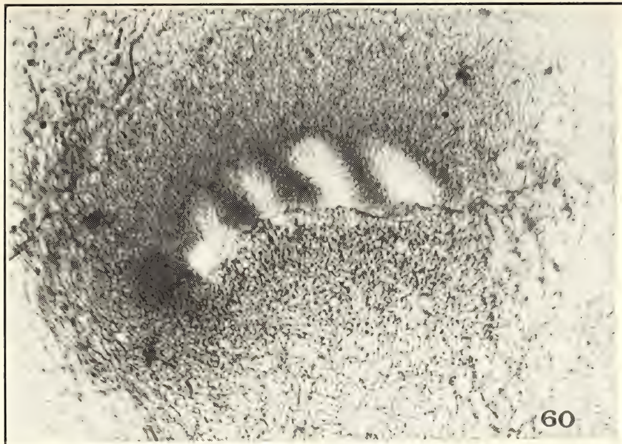
Fig. 88. Longitudinal median section of young carpophore with long, attenuated


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
48
43
42
17
18
19
20
21
22
49
23
24
25
26
27
28
29
30
31
32
33
35
36
37
38
39
34
46
41
47
51
44
45
50
40

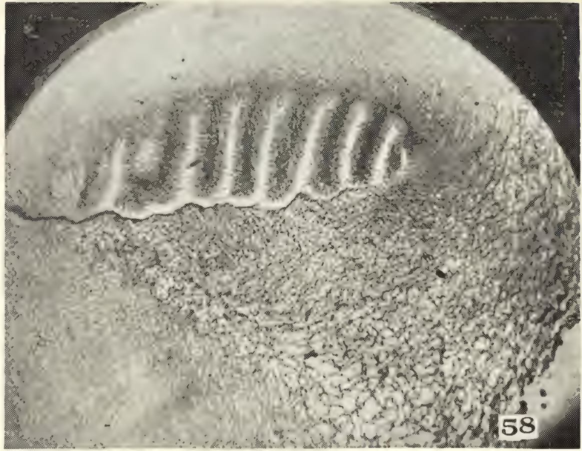
Levine: Development of Lamellae


52
53
A
B
54
55
56
A
57

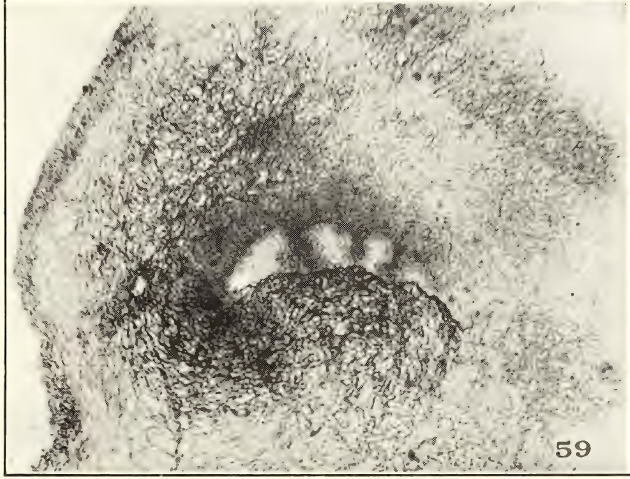
LEVINE: DEVELOPMENT OF LAMELLAE



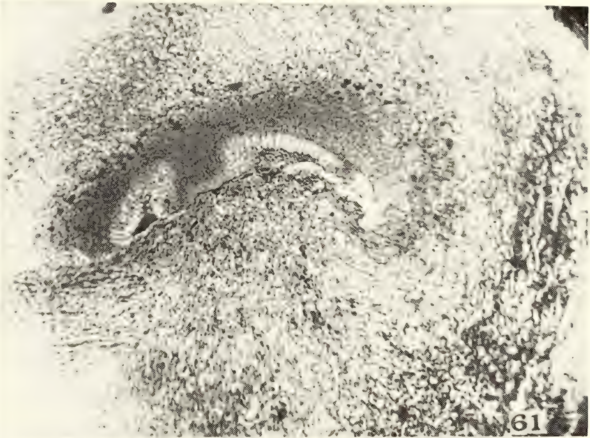
60



58

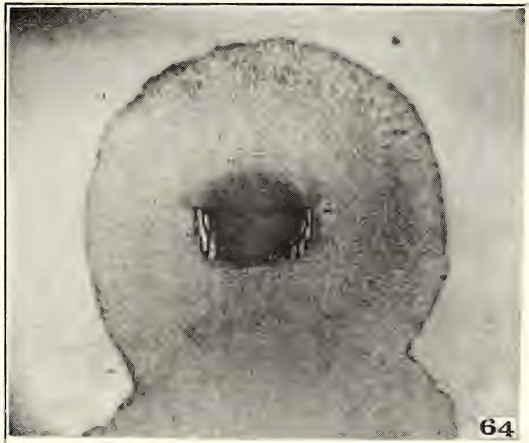


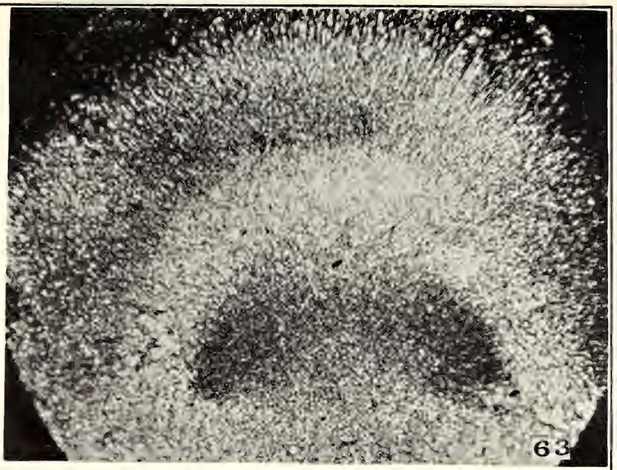
59

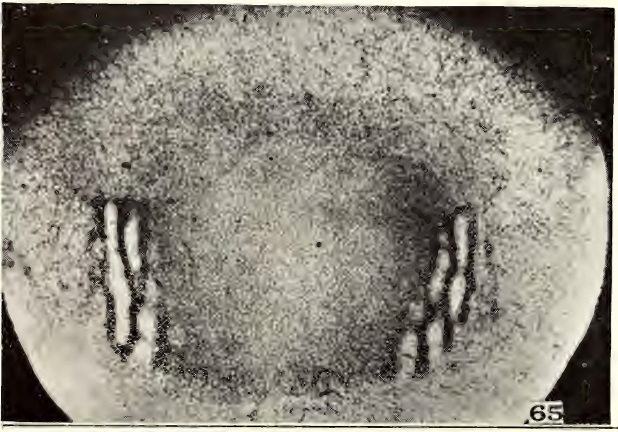


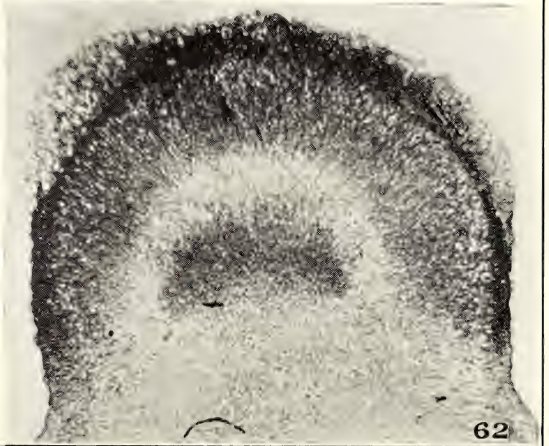
61

LEVINE: DEVELOPMENT OF LAMELLAE

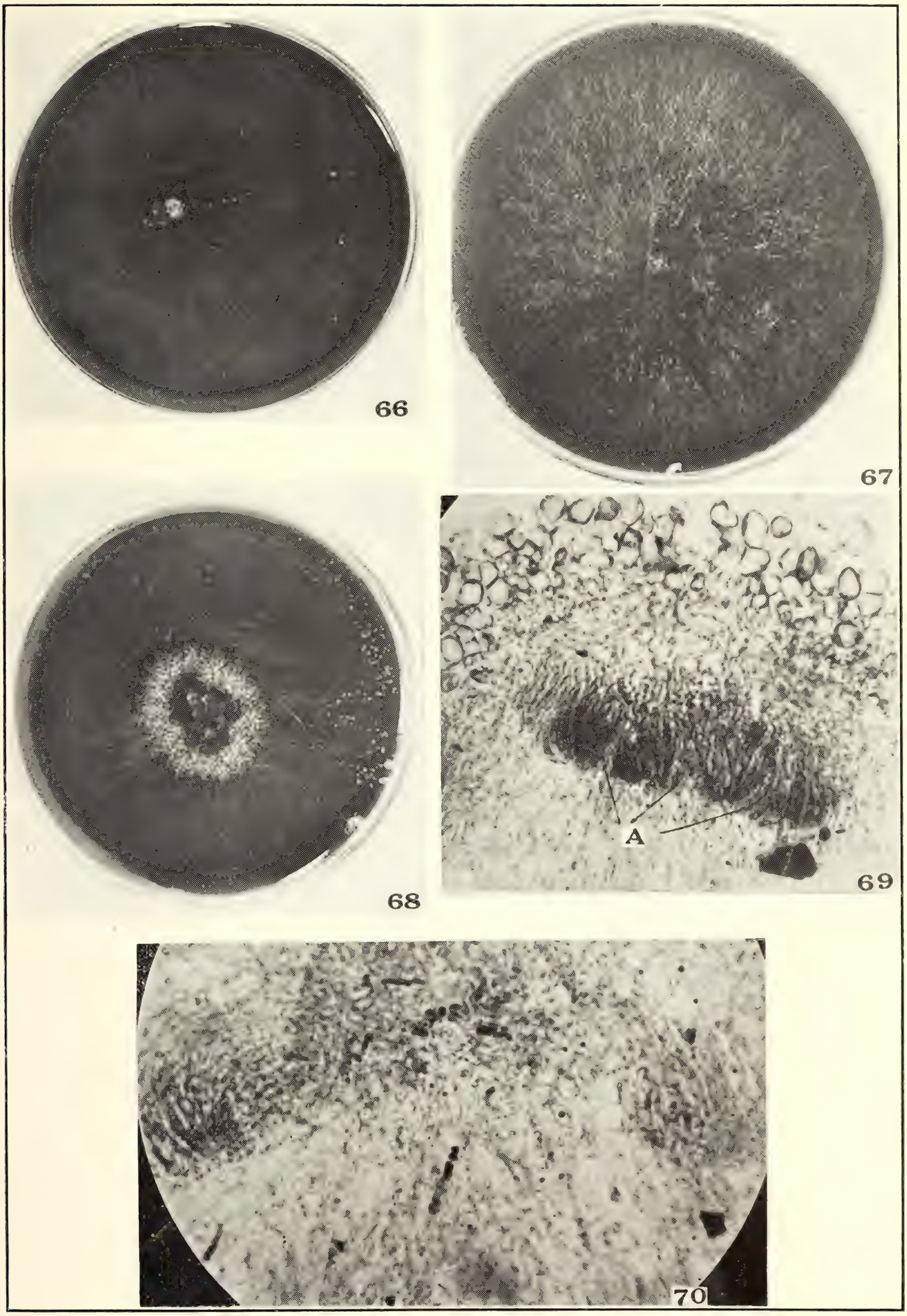




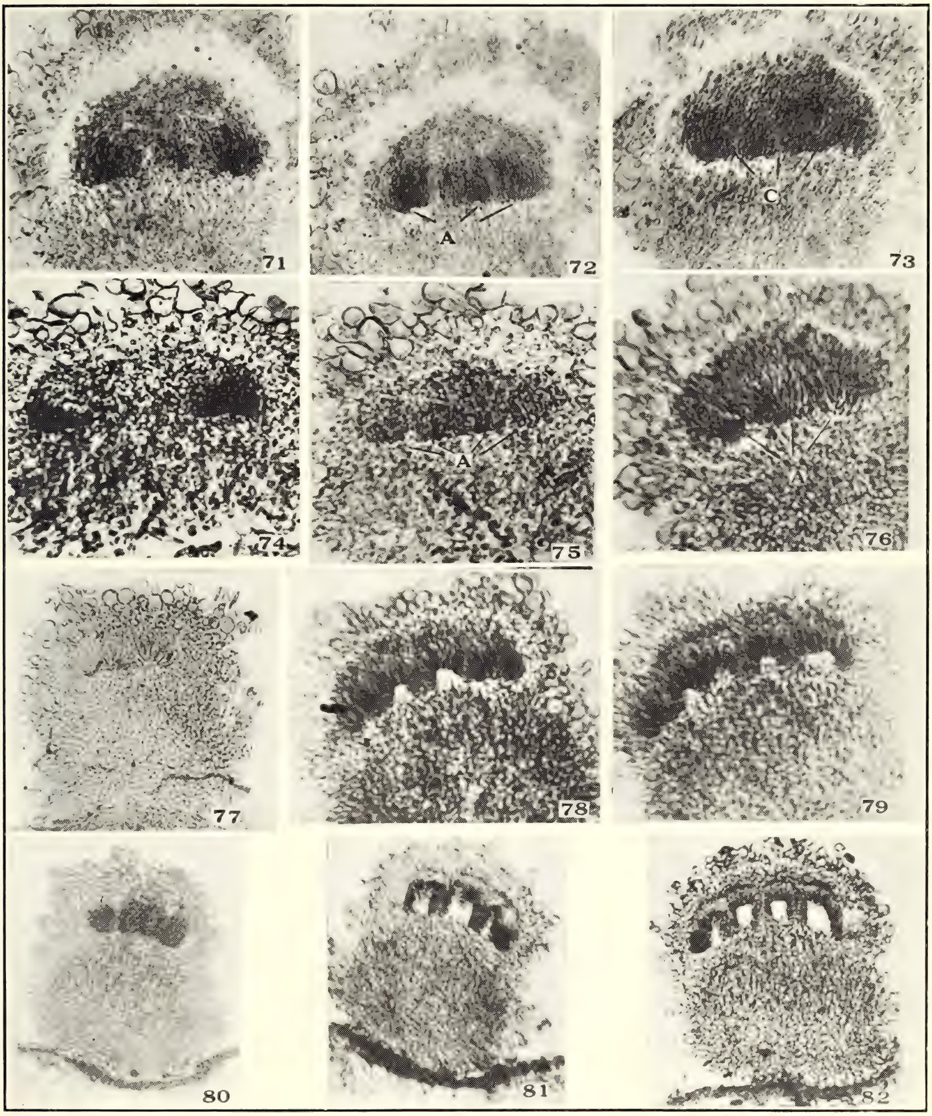




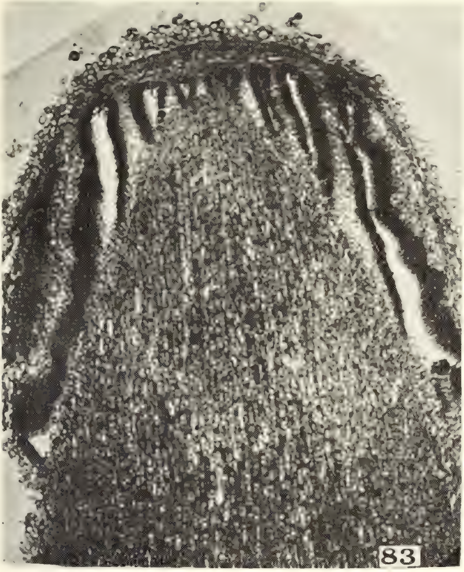
LEVINE: DEVELOPMENT OF LAMELLAE


66
67
A
69
68
70

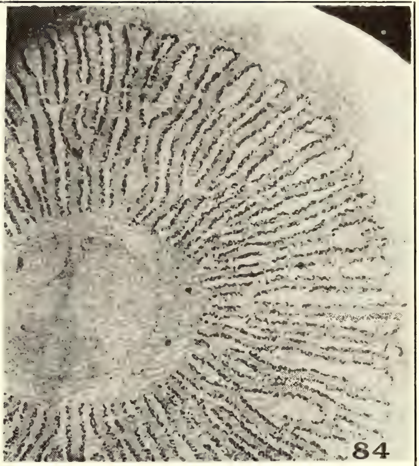
LEVINE: DEVELOPMENT OF LAMELLAE


71
A
72
C
73
74
A
75
A
76
77
78
79
80
81
82

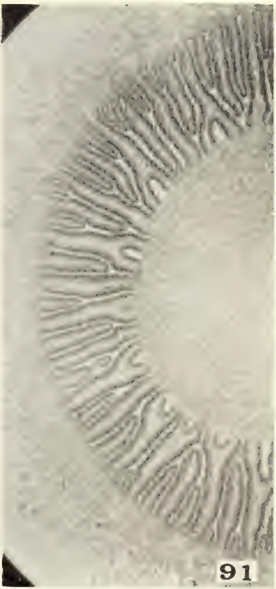
LEVINE: DEVELOPMENT OF LAMELLAE



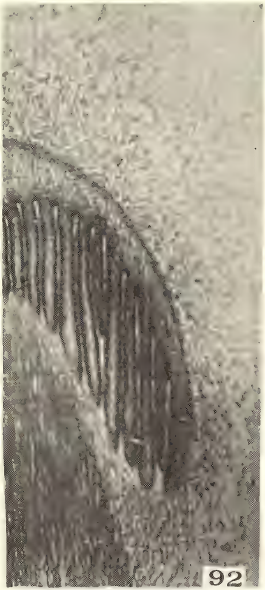
83



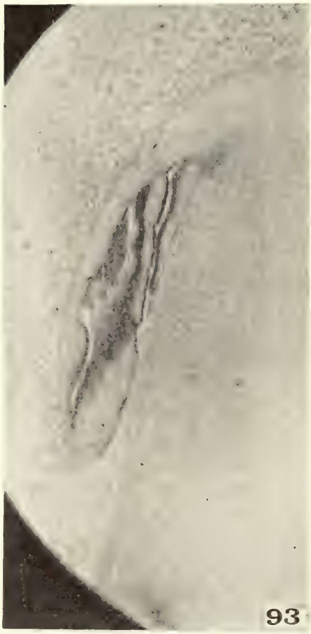
84



91

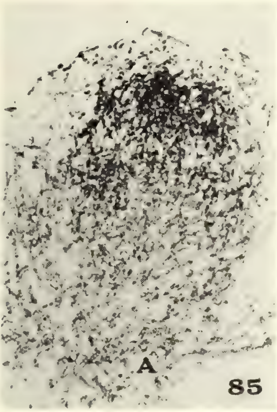


92

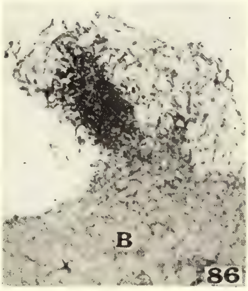


93

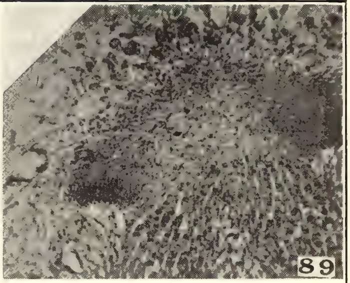
LEVINE: DEVELOPMENT OF LAMELLAE



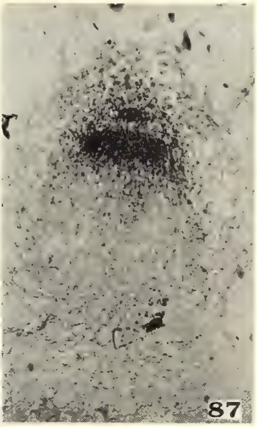
85



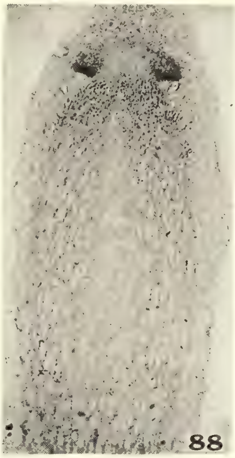
86



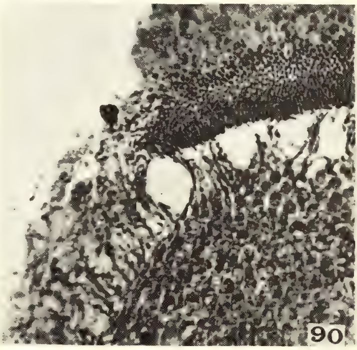
89



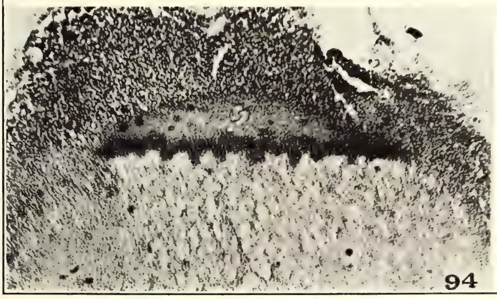
87



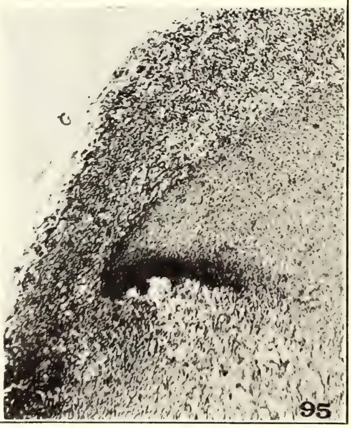
88



90



94



95

LEVINE: DEVELOPMENT OF LAMELLAE

stipe and a pilear region with no true lamellae forming. Ocular 3, objective 3, bellows 20 cm.

FIG. 89. An enlargement of a portion of the pileus shown in figure 88, showing a smooth hymenial surface. Ocular 3, objective 6, bellows 20 cm.

FIG. 90. Longitudinal tangential section of a similar button, showing a smooth hymenial surface with some of the fundamental tissue hyphae of the stipe continuous with the hyphae of the pileus. Ocular 3, objective 6, bellows 20 cm.

FIGS. 94, 95. Longitudinal tangential (fig. 94) and median (fig. 95) sections of a young button of *C. atramentarius*, showing the trama of the young gills continuous with the fundamental tissue of the stipe below. Ocular 1, objective 3, bellows 20 cm.

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1922

President: Henry C. Cowles
University of Chicago

Vice-President: Margaret C. Ferguson
Wellesley College

Secretary: I. F. Lewis
University of Virginia, University, Va.

Treasurer: Irving W. Bailey
Bussey Institution, Harvard University

Councilors:

N. L. Britton
New York Botanical Garden

J. C. Arthur
Purdue University

C. E. Allen
University of Wisconsin

Any one actively interested in botanical work is eligible for membership. Candidates for membership must be recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including one subscription to the American Journal of Botany. Candidates proposed for membership, and approved by the Council, may receive one copy of the Journal at $4.00 per year pending their election at the winter meeting of the Society.

A Fair And Frank Way To Buy Your Greenhouses

You and I know full well that salesmen have a way of putting the best foot foward, so to speak. In which particular, ours are quite like the rest. They honestly believe we build the best that's built.

Their belief, however, is not based so much on what we have told them as on what they have seen of our houses and our way of doing things.

What they really sell is their confidence in Hitchings & Company.

After which the buyer himself sells the greenhouse to himself.

The shortest short cut to that point of satisfaction, is to go and see some Hitchings houses and talk with their owners.

With that in mind let us send you names of some nearby houses.

We will gladly go with you, however, that is as you may incline.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

PHILADELPHIA
133 So. 12th St.

Issued Jan. 1923 (Sect. 1 vol. 10)

Vol. IX. DECEMBER, 1922 No. 10

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

CONTENTS

The effect of Bordeaux mixture upon the chlorophyll content of the primordial leaves of the common bean, Phaseolus vulgaris L. . . . . W. A. Ruth 535

The effect of low-temperature storage and freezing on fruits and vegetables Lon A. Hawkins 551

Revision of the South American species of Cuscuta I. . . . . T. G. Yuncker 557

Index to Volume IX. 576

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
At Prince and Lemon Sts., Lancaster, Pa.
LONDON AGENTS
WHELDON AND WESLEY, Ltd.
2, 3, & 4 Arthur St., London, W. C. 2

Smithsonian Institution
JAN 12 1923
National Museum

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN
JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
The Thompson Institute
For Plant Research, Inc.

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

H. H. BARTLETT,
University of Michigan

ARTHUR H. GRAVES,
Brooklyn Botanic Garden

I. F. LEWIS,
University of Virginia

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Postage to Canada, 20 cents; to other foreign countries (except Mexico and Cuba) 40 cents a volume on annual subscriptions. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume, post free.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $4.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or The New Era Printing Co., Inc., Lancaster, Pa.

AMERICAN

JOURNAL OF BOTANY

Vol. IX DECEMBER, 1922 (9 [illegible] No. 10

THE EFFECT OF BORDEAUX MIXTURE UPON THE CHLOROPHYLL CONTENT OF THE PRIMORDIAL LEAVES OF THE COMMON BEAN, *PHASEOLUS VULGARIS* L.

W. A. Ruth

(Received for publication March 4, 1922)

Introduction

To the quantitative relations and specific function of chlorophyll is to be attributed the faculty of the plant to produce much or little organic material under the prevailing conditions. Our knowledge does not enable us to make a final statement concerning the difference in chlorophyll in different individuals or species, but the results of recent and earlier investigations would lead us to the belief that the difference in performance, that is, in the ability to produce organic matter, rests largely, if not entirely, with the protoplast and depends upon the quantity, rather than the quality, of chlorophyll.

Until Willstätter and Stoll (1913) published a method of analysis, the absolute quantity of chlorophyll per unit area of leaf tissue could not be determined. Thus, Rosé (1913, p. 29) compared leaf extracts with a standard extract of unknown value.

> La chlorophylle n'ayant pas été isolée, on ne peut, comme le fait observer Lubimenko, procéder à des déterminations de quantités absolues. Il faut se contenter de la détermination de quantités relatives à un étalon.

A plan for a systematic quantitative study of the chlorophyll content of foliage leaves is part of a general project of the laboratory of plant physiology of the University of Illinois. This paper is limited, for the most part, to a study of the effects of Bordeaux mixture; other relations were studied more or less incidentally, these consisting of the relation of the amount of chlorophyll to the age of the leaves and to the rapidity of their growth.

The studies are to be prosecuted along two general lines: First, a study of the quantitative relation of the chlorophyll content of plants treated in various ways but growing under the general external conditions which

[The **Journal** for November (9 : 471–533) was issued November 27, 1922].

practical greenhouse men now advise for vegetable growing. Such conditions, in the main, approach very closely the ecological optimum (Schimper, 1903, p. 44). Second, a study of the chlorophyll content of leaves as affected by the shifting of one or more of the ecological factors, as light, temperature, or moisture, independently or together, in a plus or minus direction, etc. The second plan involves long and difficult experimentation if the results are to be of permanent value. It becomes necessary to provide for the strict regulation of each environmental factor or factors working together or in opposition. The variation in effect of a single factor under widely varied conditions has been clearly brought out by Livingston (1917*a*, p. 6). The work reported in this paper is based on the former method. I wish to thank Professor Charles F. Hottes, of the Department of Botany of the University of Illinois, for his advice and help, which he gave me at every stage of this work.

It is a well-established fact that a large number of plants sprayed with Bordeaux mixture show a distinctly greener color. This would lead one to expect an increase in the chlorophyll content of sprayed leaves. The deeper green of sprayed as contrasted with unsprayed leaves might be brought about by an increase in the quantity of chlorophyll in the plastids, or by an increase in the number of plastids, or in both ways. It might, on the other hand, be due to a smaller size of individual cells, and consequently to the crowding of the normal number of plastids into a smaller area. To my knowledge no quantitative experiments have been made to measure the increase in chlorophyll content of sprayed leaves, if such an increase takes place.

That the various effects of Bordeaux mixture are due to the copper ion has been the conclusion of most, but not of all, investigators. Frank (1888, p. 535) found that distilled water had a toxic effect upon the roots of lupines. Loew (1891) attributed the poisonous properties of distilled water to traces of copper in solution. Nägeli (1893) found that water in a glass vessel in which copper coins had been placed had a toxic effect upon Spirogyra. Because of the extreme dilution he thought that the action of the copper was not chemical, but due to some other force, which he described as "oligodynamic." Rumm (1893), because he was unable to find traces of copper in leaves affected physiologically by Bordeaux mixture, and Frank and Krüger (1894), because they thought that too little copper was in solution in the fungicide to act on fungous spores, applied Nägeli's hypothesis of oligodynamic action. Millardet and Gayon (1885) found that the normal germination of spores of *Peronospora viticola* would not take place at a higher concentration of copper sulphate than three parts in ten million. They found later (1887) that the cuticle of leaves had the power of removing copper from a solution of copper suphate; they believed that copper was taken out of solution in this way from Bordeaux mixture and actually penetrated the cells. Crandall (1909, p. 230) objects to the methods of

Millardet and Gayon because of the large amounts of soluble copper they used in their experiments and the insolubility of the Bordeaux precipitate. Pickering (1910, pp. 113–115) concludes from his experimental work that penetration takes place, and that the carbon dioxide of the air is the dissolving agent (1910, pp. 27–36). Bain's very careful investigations (1902) have sustained the hypothesis of penetration. Bain (1902, p. 88) states his conclusions as follows:

> From all the evidence presented on the preceding pages, there can remain little doubt that copper is absorbed by the leaves of plants sprayed with Bordeaux mixture. . . . Of course, the most conclusive and only final evidence of the entrance of the copper into the tissues of the leaf is to demonstrate its presence there by an appropriate test. The writer has made no experiments in this direction.

The belief of Millardet and Gayon in the actual penetration of copper receives further support from Schander (1904) and Ewert (1905). Schander believed, however, that the action of rain and dew is seldom of direct importance in dissolving copper salts from Bordeaux mixture because of the insolubility of the precipitate, and that in many plants, *e.g.*, in the bean and apple, the entrance of copper occurs through the hairs as the result of their excretion of an alkaline substance, or through glands in the case of other plants, like Fuchsia and Oenothera, following the excretion of acidic substances.

That the physiological effects of Bordeaux mixture are due to the penetration of copper, not only in solution but in solution in the ionic state, seems very probable if we consider the indirect evidence supplied by the work of Kahlenberg and True (1896) and Heald (1896), in connection with that of Bain (1902), Clark (1902), and Pickering (1908, 1910). Kahlenberg and True, and Heald, working with seedlings in very dilute solutions of copper salts, proved that the toxicity of the dissolved copper salt was directly proportional to the concentration of copper ions in the solution. Bain (1902, pp. 42–52) showed that sprayed peach foliage is not injured unless liquid water is present on the leaf. One would expect these results in view of the probable effect of the above-named substances in increasing or decreasing the concentration of copper ions in the Bordeaux suspension. Bain also showed (1902, pp. 36–44) that certain salts, for example, calcium chloride, calcium sulphate, and calcium nitrate, produce an increased Bordeaux injury, while lime and various carbonates have a tendency to prevent it. Clark (1902) and Pickering (1910, pp. 143–145) have shown that potassium sulphate decreases the toxicity of copper sulphate, and Pickering (1908, p. 106) has shown that sodium chloride increases it. Potassium sulphate and sodium chloride would modify the ionization of solutions of copper sulphate in a manner corresponding to the decreased and increased injury.

Besides the change in the concentration of copper ions, antagonisms, such as that of copper and calcium which has been demonstrated by True and Gies (1903) and Hawkins (1913), may have been involved in the effect of additions of other salts. As a result of the work of Boussingault (1878) and Bain (1902), there can be little doubt that the relatively insoluble calcium salts found in Bordeaux mixture can penetrate the leaf.

Duggar and Bonns (1918), in a study of the effects of spraying with Bordeaux mixture on transpiration, offer as an explanation of its effect on this particular process an entirely different hypothesis: The Bordeaux mixture, which Duggar and Cooley (1914) regard as a film, acts as a bibulous material, taking water directly from the interior of the plant. This explanation is based on the facts, as observed by them, that xerophytic plants show no increased transpiration when sprayed, and that the increase occurs, in the case of mesophytes, at night only, at which time the stomata are probably suffused with water.

The manner of action of Bordeaux mixture has remained unsolved. The importance of the fact that the presence of the precipitate in contact with the solution assures a constant supply of dissolved copper salts has been brought out by Pickering (1910, pp. 6–9). The low concentration can not play the part in Bordeaux mixture that it plays in the case of a solution not in contact with the solid phase, where the concentration, as Clark (1902) has shown, is important. This fact should overcome some of the objections to the penetration theory.

Materials and Methods

Plants of *Phaseolus vulgaris* (variety Dreer's Extra Early Refugee) were used in all the experiments. The seed was obtained in one lot from Vaughan's Seed Store, Chicago, Illinois. The plants were grown in the greenhouse, in a uniformly mixed and sifted Urbana brown silt-loam, in flats three inches deep, ten and one half inches wide, and twenty-two and one half inches long (inside measurements).

In planting, the slightly moist soil was sifted into the flats and all soil within one inch of the top was removed. The beans were laid on this soil two inches apart in the row and with four inches between the rows. One inch of soil was then added, smoothed off, and evenly compacted. The flats were watered and covered until the seedlings appeared above the soil, and the latter were then transferred to rotating tables similar to those described by Livingston (1917*b*, p. 149).

The rotating tables were located in the center of a south room in the greenhouse. The tripods were set on widely spaced two-inch boards, which were supported on a solid frame made of iron pipes. The nature of the foundation and the height of the platforms above the ground (four feet) secured a free circulation of air between the flats, and, together with the distance from the heating pipes (six feet), guarded against uneven heating and uneven relative humidity. Power was transmitted from the motor to the tables by a belt. The motor was located on a separate stand to reduce vibration. The period of rotation of the tables was six and one half minutes.

The steam heat of the greenhouse was under thermostatic control, maintained at twenty degrees C. The humidity of the air was controlled by flooding the broad trenches under the slatted walks and benches each morning. At this time the flats were watered. On cloudy days the tables were artificially illuminated by stage flood lights, each of which was provided with a 1000-watt nitrogen-filled tungsten lamp. The distance from the lamps to the tables was five feet. The effectiveness of this additional illumination as an aid to the normal growth of the plants (Lubimenko, 1905) is indicated by the prevention of unusual elongation of the stem on such days. The results obtained by this artificial lighting may be illustrated by the following: December 13, 15, and 16 were very cloudy. On December 13, the cotyledons of the young plants were just appearing above the soil of two flats. One of these was artificially illuminated by means of a flood light; the other, similarly located, received daylight only. The length of the stem to the primordial leaves of the former averaged 9 cm. on December 16 (individual measurements, 10, 9, 13, 10, 9, 9, 6, 6, 6, 12, 9, 6, 11, 9, and 9 cm.), while the stems of the latter averaged 15 cm. (individual measurements, 16, 16, 13, 16, 15, 14, and 16). (*Cf.* Johnston, 1917.)

As soon as the primordial leaves had unfolded the plants were thinned out. From twelve to twenty-four plants of uniform appearance and growth, and as evenly spaced as possible, were left in each flat. In the experiments on the effect of spraying with Bordeaux mixture, the plants in one half of each flat were sprayed; in the experiments on the relation of the chlorophyll content to the rapidity of growth of the primordial leaves, disbudding, by which the increased rate of growth of the primordial leaves was secured, was carried out on alternate plants. In the experiments on the effect of age, care was taken to maintain an equal spacing as plants were removed during the progress of the experiment.

The results recorded are based upon data secured through the use of the primordial leaves only. No selection of plants or leaves was made at harvesting. The leaves were measured and weighed in the fresh condition and immediately dried. The areas were determined by drawing the outline of the leaves on weighed letter-size sheets of Hammermill Bond paper, and weighing the enclosed portion of the sheet. The paper was found to be remarkably uniform in weight; nevertheless, each sheet was weighed separately. The leaves were dried in a rapid current of air at a temperature of from forty-five to fifty degrees C. About an hour was required for average-sized leaves. During drying, the leaves were protected from sunlight. As soon as the leaves were crisp, each lot was stored in a small tin box until its chlorophyll content was determined.

The manner of determining chlorophyll consisted in the comparison in a Duboscq colorimeter of 85-percent acetone extracts made from 0.25-gram samples of these leaves after thorough powdering in a mortar, with a standard extract made in the same manner from a bean-leaf powder which had been standardized by quantitatively determining its chlorophyll content.

The quantitative determination of chlorophyll by the use of these raw acetone solutions, which contained all four pigments, chlorophyll *a*, chlorophyll *b*, carotin, and xanthophyll, was possible because the shade of color of all the solutions closely matched that of the solution used as a standard.

> Die Rohchlorophyllösung enthält die vier Pigmente von verschiedener Farbe und Farbintensität in Mengenverhältnissen, die von Lösungsmittel beeinflusst werden und die von der Pflanzenart und sogar von der Ernte einer und derselben Pflanze abhängig sein können. *Annähernd gleich ist das Mengenverhältnis der Komponenten bei den Extrakten einer Pflanze; man kann daher durch den Vergleich derselben die relative Bestimmung ihres Chlorophyllgehaltes ausführen.* Für die Untersuchung von Extrakten ungleicher Farbnuance, also z.B. aus verschiedenenen Pflanzen, ist es erforderlich, die Farbstoffe durch Verseifung mit Alkali in die indifferenten gelben und in die Alkalisalze der grünen Pigmente zu trennen. Die erhaltenen Chlorophyllinlösungen ermöglichen die relative Bestimmung des Farbwertes und ferner erlaubt ihr Vergleich mit einer alkoholischen Lösung von bekanntem Chlorophyllgehalt die absolute Bestimmung des Farbstoffgehalts. (Willstätter and Stoll, 1913, pp. 78, 79.)

The primordial leaves used in making the standard powder were obtained from beans grown in the greenhouse from the same lot of seeds used in growing the plants for the experiments.

In carrying out the quantitative determination of chlorophyll in the powder to be used as a standard, an indirect process is necessary, because mechanical difficulties interfere with quantitatively separating chlorophyll as a solid. The process is, briefly, as follows: Chlorophyll (the mixture of chlorophyll *a* and chlorophyll *b*) is obtained from a rather large but not exactly weighed quantity of the powder and dried. A quantitatively weighed amount of this chlorophyll is dissolved in absolute alcohol and made up to a definite volume. A quantitatively weighed amount of the leaf powder from which the chlorophyll has been obtained is extracted, the carotin and xanthophyll are removed from the extract, and the resulting solution, made up to a definite volume (in water and alcohol), is compared colorimetrically with the solution made directly from the weighed chlorophyll. To facilitate removing carotin and xanthophyll from the extract from the powder, the chlorophyll *a* and chlorophyll *b* are saponified, giving the corresponding chlorophyllins (chlorophyllin *a* and chlorophyllin *b*) in unchanged proportion. The saponification and resulting comparison of chlorophylls and chlorophyllins does not introduce an error, according to Willstätter and Stoll (1913, p. 82).

To isolate the chlorophyll the following steps are necessary: (1) complete extraction of the leaf powder with eighty-five percent acetone; (2) transference of the pigments to petrol ether; (3) removal of the greater part of the acetone by washing with water; (4) separation of xanthophyll by washing with eighty percent methyl alcohol; (5) removal of acetone and methyl alcohol by washing with water, which results in the precipitation of the chlorophyll; (6) removal of the water by means of anhydrous sodium sulphate; (7) removal of the fine precipitate of chlorophyll and its separation from carotin by filtering through talc; (8) solution of the precipitated chlorophyll in ether and reprecipitation by adding petrol ether; (9) filtration through talc; (10) solution of the precipitate in ether; (11) evaporation to dryness. (The processes are described and discussed in detail by Willstätter and Stoll, 1913, pp. 132-135.)

The chlorophyllin solution was made by (1) transferring the pigments in a raw eighty-five-percent acetone extract to ether; (2) saponification of the two chlorophylls with methyl-alcoholic potash; (3) the separation of these products from carotin and xanthophyll by extracting them with water. (Willstätter and Stoll have given complete directions for this separation, 1913, pp. 81–82.)

The only modification of the method presented by Willstätter and Stoll was the use of comparatively larger quantities of solvent than the small quantities of leaf powder called for. The entire amount of leaf powder to be standardized was mixed and quartered, and from these portions were taken 0.25-gram samples for the preparation of the raw solutions in 85-percent acetone, to be used as standards, 10 grams for the isolation of the solid chlorophyll mixture, and 2.5-gram samples for the preparation of the chlorophyllins.

The samples used for chlorophyllin preparation were extracted with 85-percent acetone and made up to 100 cc. Ten cc. of this were used in the succeeding steps. A solution made by diluting the result of the final separation to 100 cc. with alcohol was of the proper strength for comparison with the standard chlorophyll solution of Willstätter and Stoll. (0.0513 gram chlorophyll is dissolved and made up to 100 cc. in absolute alcohol. For use in the colorimeter 10 cc. are diluted to 200 cc. 1913, p. 81.)

In making a raw extract for colorimetric comparison a 0.25-gram sample was extracted with successive small quantities of 85-percent acetone, which were filtered off, combined, and made up to 50 cc. in a graduated flask. Portions of approximately 2 cc. were used in the colorimeter. The depth of the standard raw acetone extract in the colorimeter was maintained at 20 mm.

Variation in Chlorophyll Content with Development of the Primordial Leaves

Before determining the effect of spraying upon the chlorophyll content, it was thought desirable to determine the degree of variation in chlorophyll content which took place as the plant grew. Willstätter and Stoll (1915) have shown that the chlorophyll content of leaves of certain species increases until the leaf is mature, after which it decreases. Possibly the decrease in chlorophyll may be attributed to the breaking down of the proteins of the chloroplasts, due to a shortage of carbohydrates in the plant and their use as a source of this material (Meyer, 1918), or to a shortage of nitrogen (Schertz, 1919). Palladin (1891) has shown definitely that a supply of soluble carbohydrates is necessary for the formation of chlorophyll, and it was thought that, aside from the age factor, the presence and dropping of the cotyledons might be involved. Their loss might mark a point of change in the chlorophyll content of the primordial leaves, caused possibly by the decreased supply of nitrogen or of carbohydrates or of both. Such changes might, it was thought, be great enough to overshadow or counterbalance any effect of spraying, although the possibility remained that they would emphasize it. Their importance in the growth of *Phaseolus vulgaris* is shown by the work of Lüpke (1888), and in the growth of the Canada field pea by Duggar (1919), who assumes that they may be a source of organic nitrogenous material or of a vitamine.

The chlorophyll content of the primordial leaves was determined at the following four stages of development: "Age *A*," immediately after the primordial leaves had unfolded; "Age *B*," when the cotyledons were being shed, three days after "Age *A*"; "Age *C*," four days after "Age *B*," when the primordial leaves had attained a considerable size, but while their area still constituted the greater part of the photosynthetic surface of the plant; and "Age *D*," when the primordial leaves constituted only a small part of the total leaf surface but before they showed yellowing. Seventeen days elapsed between "Age *C*" and "Age *D*."

The data for "Age *A*" were obtained by measuring and weighing the primordial leaves of thirty-two plants; the primordial leaves from twenty plants were used to obtain the data for "Age *B*"; those from sixteen plants for "Age *C*"; and those from twenty plants for "Age *D*." The results are shown in table 1.

TABLE 1. *Chlorophyll Content of Primordial Leaves at Various Ages*

Age	Area Both Primordial Leaves		Fresh Wt. Leaf per sq. cm. (g.)	Wt. Chlorophyll (mg.)	
	Sq. cm.	% Increase		Per sq. cm.	Per g. Fresh Wt.
"*A*".....	23.4		.0241	.0305	1.26
"*B*".....	64.6	176.	.0186	.0412	2.22
"*C*".....	107.8	67.	.0201	.0395	1.96
"*D*".....	146.0	35.	.0219	.0388	1.77

During the period of growth before the cotyledons were shed, which was also the period of most rapid expansion, there was a marked increase in the amount of chlorophyll to the square centimenter. The increase to the gram of fresh weight was still more marked. From this period on there was a decrease in the amount of chlorophyll to the square centimeter and gram of fresh weight, which was associated with an increase in the fresh weight of the leaf per square centimeter and a lessened rate of increase in area. This decrease was not so great that neutralization of any effect of early spraying was anticipated before "Age *D*" was reached. The necessity was recognized of comparing plants harvested only at the same stage of development.

The correlation between rapid growth and high chlorophyll content was determined also in the following way: To accelerate and increase the growth of the primordial leaves of certain plants, the growing buds above them were kept cut off. The primordial leaves of such plants became much larger, thicker, and greener than the primordial leaves of the check plants among them. Measurements of the area and weight were made when the plants were picked, which was four days after the cotyledons were shed ("Age *C*"). The leaves from the plants similarly treated in each flat were dried, and their chlorophyll content was determined together. The figures presented in table 2 are half-flat averages. Three flats were used, in which forty-two plants were grown. A greater chlorophyll content per square centimeter was plainly produced, which was to be accounted for only partly on the basis of a greater weight of a unit area of leaf; the average increase in the chlorophyll per square centimeter was 143 percent, while the weight of the leaf per square centimeter was increased only 126 percent. An actual increase in the amount of chlorophyll in a unit weight of fresh leaf (114 percent) was thus an important factor. The average increase in chlorophyll per square centimeter (143 percent) corresponded with the average increase of 142 percent in the rate of expansion of the whole leaf in area.

TABLE 2. *Effect of Removal of Growing Buds upon Growth and Chlorophyll Content of Primordial Leaves*

Flat	Average Area Both Leaves			Average Weight Both Leaves per sq. cm.			Average Chlorophyll per sq. cm.			Average Chlorophyll per g. Fresh Wt.		
	Check	Buds Removed		Check	Buds Removed		Check	Buds Removed		Check	Buds Removed	
	Sq. cm.	Sq. cm.	% of check	G.	G.	% of check	Wt. (mg.)	Wt. (mg.)	% of check	Wt. (mg.)	Wt. (mg.)	% of check
1	76.9	119.9	154	0.0181	0.0225	124	0.0315	0.0454	144	1.74	2.02	116
2	83.2	112.1	135	0.0186	0.0241	129	0.0353	0.0524	148	1.89	2.17	115
3	93.2	127.7	137	0.0199	0.0251	126	0.0381	0.0527	138	1.91	2.10	110
Ave.			142			126			143			114

The Effect of Bordeaux Mixture

The experiments immediately following were to determine, by measuring the length of sprayed and unsprayed leaves, (1) how soon after spraying young primordial leaves, just unfolding, the effect upon growth was to be observed; (2) whether the growth rate was increased or decreased; (3) when the effect was most manifest; and (4) whether any such effect was outgrown. A quickly manifested effect was anticipated in view of the rapid growth of the leaves and the quickly manifested effect of Bordeaux mixture upon transpiration (Martin, 1916).

Five flats were planted to beans, one flat at a time, at intervals of from one to five days. The total period of planting covered eighteen days, and the plants were exposed, on that account, to slight variations in weather conditions. As soon as the primordial leaves had unfolded, the plants in one half of each flat were sprayed with Bordeaux mixture. The length of the leaves was determined at that time and at intervals of two, four, eight, twelve, and fourteen days after spraying. Measurements made on the fourteenth day gave the same average lengths as those made on the twelfth. No measurements made after the twelfth day are presented.

Although the average length of the sprayed leaves in three of the five flats somewhat exceeded the length of the leaves not to be sprayed when the experiment was started, the sprayed leaves in all the flats were the shorter at the conclusion of the experiment. The effect in decreased growth was plainly evident on the second day, and probably could have been observed earlier. On the fourteenth day the relative differences to be observed on the second day had not been overcome. The results are summarized in table 3.

Table 3. *Effect of One Application of Bordeaux Mixture as Shown by a Lessened Growth in Length*

Days after Spraying[1]	Treatment	Average Length Primordial Leaves (cm.), Flat Number					Relation of Length of Sprayed to Unsprayed Leaves in Percentages
		1	2	3	4	5	
0	Sprayed	6.1	4.3	4.6	3.9	3.3	103
	Unsprayed	6.1	4.1	4.4	3.6	3.4	
2	Sprayed	6.8	5.4	5.9	5.5	4.8	97
	Unsprayed	7.1	5.6	5.9	5.8	4.9	
4	Sprayed	7.2	6.4	6.8	6.6	5.9	96
	Unsprayed	7.7	6.5	6.9	6.9	6.3	
8	Sprayed	7.6	7.0	7.6	7.7	6.3	96
	Unsprayed	8.2	7.2	7.8	7.8	6.5	
14	Sprayed	7.7	7.3	7.7	7.9	7.2	95
	Unsprayed	8.3	7.4	7.9	8.3	7.8	

---

[1] The state of development of the plant when the leaves were sprayed is represented by "Age *A*" in the preceding section; the fourth day corresponds with "Age *B*," and the eighth day with "Age *C*."

It was apparent that if Bordeaux mixture resulted in a change in chlorophyll content correlated with a decreased growth rate, one might expect such a change to be revealed soon after the spray was applied. It was concluded from the results of the above-described experiment that any differences in chlorophyll content brought about by the action of Bordeaux mixture applied early in the growth of the leaf would probably be preserved rather than materially lessened.

The effect of Bordeaux mixture upon the development of chlorophyll may be indicated by reporting an experiment in which a set of six flats, planted on the same day, was used. The primordial leaves of one half of the plants in three of these flats were sprayed as soon as they had unfolded (flats 1, 2, and 3 in tables 4–7). The same treatment was given to the primordial leaves of one half of the plants in the other three flats when the cotyledons were dropping (flats 4, 5, and 6 in the same tables), four days later. The plants in all six flats were harvested on the same day, three days after the later application, and seven days after the earlier one.[2]

At the time of spraying the average length of the sprayed leaves in all the flats was 5.7 cm., while those not to be sprayed averaged 5.9 cm. Flat averages of the areas and chlorophyll contents per square centimeter when the leaves were picked are presented in table 4.

TABLE 4. *Average Areas and Chlorophyll Content per Square Centimeter of Sprayed and Unsprayed Leaves*

Flat	Area One Leaf (sq. cm.)		Chlorophyll (mg. per sq. cm.)	
	Sprayed	Not Sprayed	Sprayed	Not Sprayed
1	48.7	63.9	.00436	.00330
2	54.7	62.4	.00375	.00401
3	57.2	53.7	.00382	.00371
4	42.6	45.9	.00442	.00399
5	61.2	63.9	.00340	.00304
6	49.6	57.6	.00363	.00356
Ave.	53.3	57.9	.00390	.00361

The chlorophyll content of unit areas of the sprayed leaves from all the flats with the exception of those from flat 2 was greater than that of the corresponding unsprayed leaves. The areas of the sprayed leaves were less than the areas of those not sprayed, with the exception of those from the plants grown in flat 3. The general results were anticipated in view of the darker color of the sprayed foliage and the stunting effect of the spray which had been manifested in the previous experiment in a lessened increase in length.

The darker color was correlated with this smaller size. In spite of the lessened rate of expansion in area brought about by spraying, the chlorophyll content of the average sprayed and of the average unsprayed leaf was practically the same, averaging 2.02 milligrams to the sprayed leaf and 2.07 milligrams to the leaf which had not been sprayed. Averages for the six flats are presented in table 5.

---

[2] This stage of development is referred to as "Age *C*" in the preceding section, where the state of unfolding is designated as "Age *A*" and that of cotyledon shedding as "Age *B*."

TABLE 5. *Average Chlorophyll Content (in Milligrams) of the Primary Leaves of Sprayed and Unsprayed Plants*

Flat	1	2	3	4	5	6	Average
Sprayed	2.12	2.05	2.18	1.88	2.08	1.80	2.02
Unsprayed	2.11	2.50	1.99	1.83	1.94	2.05	2.07

This was not due to an increase in the weight of the sprayed leaf per unit area, as table 6 shows.

TABLE 6. *Weight of Sprayed and Unsprayed Leaves (in Grams) per Square Centimeter*

Flat	1	2	3	4	5	6	Average
Sprayed	.0204	.0187	.0187	.0164	.0185	.0189	.0186
Unsprayed	.0189	.0204	.0195	.0157	.0189	.0193	.0189

The primordial leaves of the sprayed plants in all the flats contained the greater amount of chlorophyll per gram of fresh leaf. The data are presented in table 7.

TABLE 7. *Chlorophyll Content (in Milligrams) per Gram, Fresh Weight*

Flat	1	2	3	4	5	6	Average
Sprayed	2.14	2.00	2.04	2.30	1.82	2.00	2.05
Unsprayed	1.75	1.97	1.96	2.17	1.61	1.86	1.89

DISCUSSION

Probable Relations between Photosynthesis and the Increased Chlorophyll Content. It has been shown that the chlorophyll content of unit areas and of unit fresh weights has been increased by spraying rapidly growing leaves with Bordeaux mixture. This increase is accompanied by a smaller leaf area. No experiments were carried out to determine the effect upon photosynthesis; this is one of a series of topics outlined for further experiment. Amos (1907), experimenting with leaves of the hop, grape, and Jerusalem artichoke, found that Bordeaux mixture temporarily checked photosynthesis After the effect of the temporary check had disappeared, photosynthesis became normal but did not exceed that of unsprayed leaves. No effect of the spray on the color of the leaves was reported, which prevents the direct application of his conclusions to this experiment.

If whole leaves had been taken as units, and if an examination had been made at "Age *C*," it is possible that the photosynthetic activity of the sprayed leaves would have been found to be about the same as that of unsprayed leaves, because the chlorophyll content of the sprayed and unsprayed leaves was approximately equal. On the other hand, the effect of the Bordeaux mixture upon other conditions involved in photosynthesis might prove such an assumption to be groundless. In fact, it is more probable that photosynthesis was decreased; at any rate, in the experiment reported above on the effect of disbudding, a direct relation was shown between a high chlorophyll content of unit areas and a rapid increase in the area of the whole leaf, while the same positive relation between the chlorophyll content and the growth rate held at the various ages. Moreover, the decreased respiration produced by Bordeaux mixture under conditions similar to those of this experiment (Ewert, 1905) might indicate a lowered photosynthetic activity, since a positive relation between photosynthesis and respiration, except under extreme conditions, has been proven by Spoehr and Long (1919). Plester (1912) concludes that structure, which is evidently changed by Bordeaux mixture, is a factor in the rate of photosynthesis of varieties of the same species differing in their chlorophyll content, and Rosé (1913, p. 105) arrives at the same conclusion as the result of his work on the photosynthetic rate manifested by leaves developed under different conditions of illumination. Another factor which might be affected is the activity of an enzym to which Willstätter and Stoll (1915) attribute the high photosynthetic activity of very young, etiolated, and chlorotic leaves in which the photosynthetic rate is much above the rate indicated by their chlorophyll content. Certain investigators, including Frank and Krüger (1894) and Bain (1902), have found greater amounts of starch in the leaves of plants treated with relatively insoluble copper compounds, including Bordeaux mixture. These investigators have concluded that photosynthesis has been increased. Ewert (1905), however, finds that the yield is always lowered by Bordeaux mixture, whether it is measured in starch, in protein, or in dry weight. He finds that respiration is decreased and the rate of translocation of carbohydrates is lessened, the latter effect accounting for the apparent increase in assimilation observed by earlier investigators. He attributes the decreased growth which he obtained to a poisoning exerted on various activities of the plant, including diastatic activity.

Aside from the harmful effect upon photosynthesis, which Bordeaux mixture may have in ways like those above suggested, it would seem that the increase in transpiration which it evidently brings about would be harmful to growth, unless the water content of the growing cells is maintained, possibly by an increased activity in water absorption by the root.

Environmental Conditions. The conditions for the growth of the plants used in this experiment were extremely favorable to rapid and normal development. Extremes were avoided, and it is probably due to the care exercised that injuries such as those reported by Frank and Krüger (1894) were not observed. These authors reported a severe Bordeaux injury to the foliage of potatoes as the result of adverse conditions, including very high temperatures. Bain (1902) also states that high temperature is a factor in the physiological response of peach foliage to copper salts, being manifested in both increased assimilation and increased injury. Crandall (1909) states that low vitality is a factor in the production of injury to the foliage of the apple following the application of Bordeaux mixture. The freedom of the plants from injury may have been due in part to the fact that the foliage was not wetted, which, it is frequently stated, is a necessary condition, although Schander (1904) has shown that bean foliage can be injured by Bordeaux mixture without the presence of liquid water. Under conditions brought about by too low temperatures or too little illumination it is possible that the leaves of the bean would show no appreciable response.

Summary

1. The primordial leaves of the bean sprayed with Bordeaux mixture do not grow to the size attained by unsprayed leaves.
2. A retardation in growth is manifested very soon after the spray is applied. The relative difference is maintained.
3. The chlorophyll content per unit area of the primordial leaves of the bean is slightly increased by spraying with Bordeaux mixture.
4. The chlorophyll content per unit area of the primordial leaves of the bean decreases as the leaves develop after the shedding of the cotyledons.
5. The chlorophyll content per unit area of the young primordial leaves of the bean is lower than that of the primordial leaves of the bean just after the shedding of the cotyledons.
6. The primordial leaves of the bean increase in area and in weight per unit area following the suppression of the growing buds.
7. This increase in area and weight per unit area is accompanied by a disproportionately greater increase in the chlorophyll content per unit area.

BIBLIOGRAPHY

Amos, A. 1907. The effect of fungicides upon the assimilation of carbon dioxide by green leaves. Jour. Agr. Sci. **2**: 257–266.

Bain, S. M. 1902. The action of copper on leaves. Tenn. Exp. Sta. Bull. **15**^{2}: 1–108.

Bell, J. M., and Taber, W. C. 1907. The action of lime in excess on copper sulphate solutions. Jour. Phys. Chem. **11**: 632–638.

Boussingault, J. 1878. Étude sur les fonctions physiques des feuilles. Annales Chim. Phys. V, **13**: 289–394.

Clark, J. F. 1902. On the toxic properties of some copper compounds with special reference to Bordeaux mixture. Bot. Gaz. **33**: 26–48.

Coupin, H. 1898. Sur la toxicité du chlorure de sodium et de l'eau de mer e l'égard des végétaux. Rev. Gén. Bot. **10**: 177–190.

Crandall, C. S. 1909. Ill. Exp. Sta. Bull. 135.

Duggar, B. M. 1919. Nutritive value of food reserve in cotyledons. Yearbook Carnegie Inst. Wash. **18**: 81, 82.

——, **and Bonns, W. W.** 1918. The effect of Bordeaux mixture on the rate of transpiration. Ann. Mo. Bot. Gard. **5**: 153–176.

——, **and Cooley, J. S.** 1914. The effect of surface films and dusts on the rate of transpiration. Ann. Mo. Bot. Gard. **1**: 1–22.

Ewert, R. 1905. Der wechselseitige Einfluss des Lichtes und der Kupferkalkbrühen auf den Stoffwechsel der Pflanze. Landw. Jahrb. **34**: 233–310.

Frank, B. 1888. Untersuchungen über die Ernährung der Pflanze mit Stickstoff. Landw. Jahrb. **17**: 420–554.

——, **and Krüger, F.** 1894. Ueber den Reiz, welchen die Behandlung mit Kupfer auf die Kartoffelpflanze hervorbringt. Ber. Deutsch. Bot. Ges. **12**: 8–11.

Galloway, B. T. 1890. Report of the chief of the section of vegetable pathology, U. S. Dept. Agr., for the year 1889.

Hawkins, L. A. 1913. The influence of calcium, magnesium and potassium nitrates upon the toxicity of certain heavy metals toward fungus spores. Physiol. Res. **1**: 57–92.

Heald, F. D. 1896. The toxic effect of dilute solutions of acids and salts upon plants. Bot. Gaz. **22**: 125–153.

Johnston, E. S. 1917. Seasonal variations in the growth-rates of buckwheat plants under greenhouse conditions. Johns Hopkins Univ. Circ. n. ser. **3**: 211–217.

Kahlenberg, L., and True, R. H. 1896. On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gaz. **22**: 81–124.

Livingston, B. E. 1917*a*. A quarter-century of growth in plant physiology. Plant World **20**: 1–15.

——. 1917*b*. The Department of Plant Physiology. Johns Hopkins Univ. Circ. n. ser. **3**: 133–159.

Loew, O. 1891. Bemerkung über die Giftwirkung des destillirten Wassers. Landw. Jahrb. **20**: 235.

Lubimenko, W. 1905. Sur la sensibilité de l'appareil chlorophyllien des plantes ombrophiles et ombrophobes. Rev. Gén. Bot. **17**: 381–415.

Lüpke, R. 1888. Ueber die Bedeutung des Kaliums in der Pflanze. Landw. Jahrb. **17**: 887–913.

MacDougal, D. T. 1919. Growth and hydration. Yearbook Carnegie Inst. Wash. **18**: 57–60.

Martin, W. H. 1916. The influence of Bordeaux mixture on the rates of transpiration from abcissed leaves and from potted plants. Jour. Agr. Res. **7**: 529–548.

Meyer, A. 1918. Eiweissstoffwechsel und Vergilben der Laubblätter von *Tropaeolum majus*. Festschrift zum Ernst Stahl, pp. 85–127. Jena, 1918. Abstract by W. Crocker. Bot. Gaz. **67**: 446, 447. 1919.

Millardet, A., and Gayon, U. 1885. Recherche du cuivre sur les vignes traitées par le mélange de chaux et de sulfate de cuivre et dans la récolte. Jour. Agr. Prat. **49**^{2}: 732–734.

—— ——. 1887. Jour. Agr. Prat. **51**^{1}: 123–129, 156–161.

von Nägeli, C. 1893. Ueber oligodynamische Erscheinungen in lebenden Zellen. Denkschr. Schweiz. Naturf. Ges. **33**: 1.

Palladin, W. 1891. Ergrünen und Wachstum der etiolierten Blätter. Ber. Deutsch. Bot. Ges. **9**: 229–232.

Pickering, S. U., and the Duke of Bedford. 1908. Woburn Exp. Fruit Farm, Rept. 8.

—— ——. 1910. Woburn Exp. Fruit Farm, Rept. 11.

Plester, W. 1912. Kohlensäureassimilation und Atmung bei Varietäten derselben Art, die sich durch ihre Blattfärbung unterscheiden. Beitr. Biol. Pflanz. **11**: 249–304.

Rosé, E. 1913. Énergie assimilatrice chez les plantes cultivées sous différents éclairements. Ann. Sci. Nat. Bot. IX, **17**: 1–110.

Rumm, C. 1893. Ueber die Wirkung der Kupferpräparate bei Bekämpfung der sogenannten Blattfallkrankheit der Weinrebe. Ber. Deutsch. Bot. Ges. **11**: 79–93.

Schander, R. 1904. Über die physiologische Wirkung der Kupfervitriolkalkbrühe. Landw. Jahrb. **33**: 517–584.

Schertz. 1919. Reported by W. Crocker. Bot. Gaz. **67**: 447.

Schimper, A. F. W. 1903. Plant geography upon a physiological basis (Eng. transl.). Oxford.

Spoehr, H. A., and Long, Frances. 1919. The interrelation of photosynthesis and respiration. Yearbook Carnegie Inst. Wash. **18**: 78–80.

True, R. H., and Gies, W. J. 1903. On the physiological action of some of the heavy metals in mixed solutions. Bull. Torrey Bot. Club **30**: 390–402.

Willstätter, R., and Stoll, A. 1913. Untersuchungen über Chlorophyll, Methoden und Ergebnisse. Berlin.

——. 1915. Untersuchungen über die Assimilation der Kohlensäure. Ber. Deutsch. Chem. Ges. **48**: 1540–1563.

THE EFFECT OF LOW-TEMPERATURE STORAGE AND FREEZING ON FRUITS AND VEGETABLES[1]

LON A. HAWKINS

(Received for publication March 25, 1922)

The importance of low-temperature storage and refrigerated transportation facilities for food products in the present state of our social and economic system can hardly be estimated. Enormous quantities of foodstuffs which might otherwise be unprofitably utilized or wasted outright are conserved each year by cold storage or are transported in refrigerated cars or ships from the localities where they are grown to communities where they can be utilized. Thus a diversity of diet ranging from tropical fruits to caribou flesh from the Arctic regions is made possible over a large part of the civilized world. The feeding of the large population in industrial centers is rendered much more economical, convenient, and sanitary by the proper application of low-temperature storage to food products.

The mechanical problems of refrigeration have of necessity received much attention, and methods have been perfected whereby constant low temperatures can be readily maintained at a comparatively low cost. The effect of these temperatures on the produce has not received as much attention as the problems seem to warrant. There are, of course, considerable data on the behavior of plant products under low temperatures, which have been obtained by empirical methods. These methods are probably not the best for determining the facts to be used as a basis for a great industry. With the inadequate facilities for careful experimentation and study of physiological problems, it has been the only way for obtaining much of the information urgently needed.

Although the present status of our knowledge of the effect of low temperatures on living plant products leaves much to be desired, a survey of the field of plant physiology shows that some work has been done. It is the province of this paper to take up briefly some phases of the effect of low-temperature storage on fruits and vegetables.

In the commercial storage of these products it is essential that they be kept in such a condition that that group of chemical and physical processes usually associated with living organisms and characterized for want of a better name as "life processes" or "vital activities" can proceed without interruption. At the same time, it is essential that these processes be slowed down as much as possible, so that this portion of the life cycle of the organism will not be completed too quickly and the product become unfit for food through autolytic decomposition or be broken down by the action of micro-organisms.

---

[1] Paper read at the symposium on "The Low Temperature Relations of Plants," at Toronto, December 29, 1921.

The physiological problems of commercial cold storage of plant products are, of course, somewhat complex; as in most problems in plant physiology the method of attack has been essentially from a chemical standpoint. The fundamental principle which apparently governs the relation of many of the processes which go on in plants, or which at least seems to explain their relation to temperature, was suggested by Van't Hoff.[2] This rule is that the rate of chemical reaction doubles or trebles for every rise in temperature of 10° C. It has been found that many of the processes which go on in plants take place in accordance with this rule; that is, within limits there is an acceleration of the processes if the temperature is raised and a retardation with the lowering of the temperature. For example, the rate at which CO_2 is given off and oxygen is taken up by plants, which is an indication at least of the rate of respiration, is more rapid at high than at low temperatures. This was shown by de Saussure and by many investigators since his time, working with various kinds of plants (see review by Czapek[3]). It has been demonstrated for apples by Morse,[4] while Gore[5] later carried out a series of respiration experiments on a large number of different kinds of fruits. He found that the factor for the average increase in the rate of respiration for 10° C. rise in temperature, as measured by the amount of carbon dioxide liberated, was around 2.37. From the results obtained by Gore there would seem to be no question but that there is a marked increase in respiration at the higher temperatures. The experiments were, however, only of short duration, and it is doubtful that the processes which result in the taking up of oxygen and the liberation of carbon dioxide and water had reached an equilibrium so that the true measure of the effect of temperature upon these processes could be obtained. In determining the effect of temperature on the rate of respiration, long-time experiments are to be recommended as giving a more accurate measure of this process. The work of Magness[6] indicates that at high temperatures there is an accumulation of CO_2 in fleshy fruits and vegetables and a very low pressure of O_2, while at low temperatures the ratio O_2/CO_2 within the fruit is considerably higher. This might well influence the kind of respiration, and the energy changes might at high temperatures be by intramolecular rearrangement and partial splitting rather than by a breaking down of the molecule to CO_2 and H_2O, while at low temperatures in the presence of larger amounts of oxygen the molecule might be more completely decomposed. Experiments at constant temperatures carried out for long periods will undoubtedly furnish some idea of the amount of CO_2 given off at these temperatures under the conditions existing in the tissue. Some work of this type is in progress at the present time and will without doubt add much to our knowledge of the subject. Some good, simple method of measuring more directly the energy changes involved in respiration is urgently needed.

---

[2] Van't Hoff, J. H. Studies in chemical dynamics (Eng. transl.). Pp. 286. London, 1896.

[3] Czapek, F. Biochemie der Pflanzen, 1st ed. **2**: 397–399. Jena, 1905.

[4] Morse, F. W. Effect of temperature on the respiration of apples. Jour. Amer. Chem. Soc. **30**: 876–881. 1908.

[5] Gore, H. C. Studies on fruit respiration. U. S. Dept. of Agr. Bur. Chem. Bull. 142, pp. 1–40.

[6] Magness, J. R. Composition of gases in intercellular spaces. Bot. Gaz. **70**: 308–316. 1920.

Changes in the chemical composition of the product, while not always of value in indicating the constituents used in respiration, are of interest in themselves. The effect of low temperatures on changes which take place in carbohydrates in plants is of especial interest, and a number of investigations have added to our knowledge of this subject. Müller-Thurgau[7] found that in potatoes stored at low temperatures (0°–6° C.) sugars accumulated and starch was broken down more rapidly, while if the temperature was raised (to 8°–10°) the sugar disappeared and starch was formed again. He explains these phenomena by differences in the velocity at which the various reactions involved take place and differences in the optimum temperature, and there is every indication at the present time that this explanation is valid. Appleman,[8] working with white potatoes, found that the carbohydrate changes are dependent on temperature and was able to corroborate much of Müller-Thurgau's work. Hasselbring and Hawkins[9] have shown that the carbohydrate changes in sweet potatoes are apparently similar to those in white potatoes. There is, of course, a higher sugar content in sweet potatoes, the cane sugar especially being much higher at the low temperatures. Sweet potatoes are usually stored commercially at relatively high temperatures (10°–12° C.), and at these temperatures if the roots have been properly cured they may be stored in good condition for a long time. If the temperature is lowered to 0° C., or even to 5° C., they become very susceptible to infection by micro-organisms and soon decay, especially if they are removed from the cold storage to a higher temperature. While it is not within the scope of this paper to discuss this phase of the subject, it is interesting to note the decrease in resistance of these roots at the low temperatures, which parallels the increase in sugar content. It is quite probable that these two reactions are not at all related but are merely concomitant. In the respiration experiments with sweet potatoes, it is shown that CO_2 is eliminated at a much higher rate at 30° C. than at low temperatures.

---

[7] Müller-Thurgau, H. Über Zuckeranhaüfung in Pflanzentheilen in Folge niederer Temperatur. Landw. Jahrb. 5: 751–828. 1882.

[8] Appleman, C. O. Biochemical and physiological study of the rest period in the tubers of *Solanum tuberosum*. Md. Agr. Exp. Sta. Bull. 183. Pp. 226. 1914.

[9] Hasselbring, H., and Hawkins, L. A. Physiological changes in sweet potatoes during storage. Jour. Agr. Res. 5: 331–342. 1915. Respiration experiments with sweet potatoes. Jour. Agr. Res. 5: 509–517. 1915. Carbohydrate transformations in sweet potatoes. Jour. Agr. Res. 5: 543–560. 1915.

With stored fruits which contain considerable quantities of acids, the acids seem to be utilized in respiration, especially at the low temperatures, though there may be some decrease in carbohydrate content as well. The acids as a rule break down more rapidly at high temperatures than at low. This has been shown to be the case with apples by Bigelow, Gore, and Howard[10] and by others. It is also true of grapefruit within limits. The sugar content of apples decreases very slowly at low temperatures, while with grapefruit[11] there seems to be a decrease of acidity at the low temperatures, and at high temperatures over long periods the acidity apparently increases and the sugars decrease. This, of course, could be explained as Müller-Thurgau explained the carbohydrate changes in potatoes, or on the theory that the grapefruit uses acids in respiration at low temperatures and carbohydrates at high temperatures with possibly the formation of acids. If space permitted, numerous instances could be cited illustrating this retarding of chemical processes in plants by lowering the temperature. The chemical reactions in plants apparently follow the same rules as chemical reactions *in vitro*, though it is, of course, impossible at present to duplicate, outside the plant itself, all the complex interrelated reactions which go to make up the so-called vital activities of the organism.

With a sufficiently low temperature the water, which, of course, is the main constituent of fruits and vegetables, freezes, and most of these reactions cease. Some few reactions may proceed slowly after the fruit or vegetable is solidly frozen, but most of them will take place only in liquid solution. When the organism is thawed and the water or solute is again liquid, many of the chemical reactions which were going on before freezing are resumed. In the case of most plant parts, however, there is a disorganization of the chemical and physical equilibria, and the processes are not checked and balanced as in the living plant. This, of course, leads to a breaking down and a decomposition of the tissue. The changes in the plant tissue brought about by crystallizing out of the water are discussed by Harvey,[12] Müller-Thurgau,[13] and others who have worked on this particular phase of the problem. In the work on freezing of fruits and vegetables carried on in our laboratories, freezing-point determinations, that is, determinations of the temperature at which the water crystallizes in the plant, have been made on a large number of varieties of some twenty or thirty of the different kinds of fruits and vegetables; altogether some fiteen or twenty thousand determinations have been made. In this work it was found that the freezing points of most of these fruits and vegetables lie between −.5° C. and − 2.5° C. The succulent plants, such as lettuce, cabbage, celery, and cauliflower, freeze at a considerably higher temperature than do potatoes, sweet potatoes, apples, and such produce, which have a relatively high dry weight. Some products, such as nuts, which have a very low water content, have a low freezing point. From our investigations in this field the conclusion seems obvious that all fleshy fruits and vegetables will be killed if exposed to a low enough temperature after ice crystals begin to form in the tissues. Many fruits and vegetables, however, can be cooled to temperatures below their freezing points and have the crystallization of the water actually take place within the tissues without apparent injury, and the occurrence of local injuries, that is, injuries to certain portions of the tissue, due to freezing, is quite common. An illustration of this point is found in the work of Jones, Miller, and Bailey[14] on potatoes. These writers show that a potato may be injured locally by freezing, the death of the cells being followed by browning of the cells around the fibrovascular bundles or in the parenchyma itself. The major portion of the potato, however, might remain sound for a long time. Wright and Harvey[15] and Wright and Taylor[16] corroborated these results in their work on potatoes. It is very doubtful, however, whether ice crystals are ever formed in the potato without injury to the tuber. In this respect they are different from some of the other vegetables and fruits. Apples may be frozen lightly and thawed without apparent injury. Hard, ripe Bartlett pears have been frozen solid, removed from the freezing room, and ripened normally without any indications that the tissues of the fruit had been injured by the solidification of the water. Ripe pears or immature pears of this variety may be seriously injured by freezing. With apples, slight freezing sometimes causes local discolorations around the fibrovascular bundles or dark-colored areas in the tissue. These problems are, of course, under investigation at the present time and are furnishing some interesting information.

---

[10] Bigelow, W. D., Gore, H. C., and Howard, B. J. Studies on apples. U. S. Dept. Agr. Bur. Chem. Bull. 94.

[11] Hawkins, L. A. A physiological study of grapefruit ripening and storage. Jour. Agr. Res. **22**: 263–279. 1921.

[12] Harvey, R. B. Hardening process in plants and developments from frost injury. Jour. Agr. Res. **15**: 83–111. 1918.

[13] Müller-Thurgau, H. Ueber das Gefrieren und Erfrieren der Pflanzen. Landw. Jahrb. **9**: 133–189. 1880.

In freezing fruits and vegetables, they may frequently be undercooled far below the freezing point without the formation of ice or without any apparent injury to the tissues. It has been found that potatoes could sometimes be cooled as much as 10° C. below their freezing points, if allowed to remain undisturbed, before the inception of ice formation in the tissue. In certain instances they have been cooled as much as 6° C. below their

[14] Jones, L. R., Miller, M., and Bailey, E. Frost necrosis of potato tubers. Univ. Wis. Agr. Exp. Sta. Res. Bull. 46. 1919.

[15] Wright, R. C., and Harvey, R. B. Freezing point of potatoes as determined by the thermoelectric method. U. S. Dept. Agr. Bull. 895. 1921.

[16] Wright, R. C., and Taylor, G. F. Freezing injury to potatoes when undercooled. U. S. Dept. Agr. Bull. 916. 1921.

freezing points and then warmed to temperatures above the freezing point without any evidences of injury. The potatoes, of course, were maintained undisturbed during these experiments.

A slight jar, such as would be caused by the slamming of the door of the freezing room, tapping the tubers gently with a lead pencil, or dropping them a short distance, promotes the inception of ice formation very readily in undercooled potatoes, and when once freezing begins, injury to the tissues is apparently sure to follow. Apples may be undercooled to a marked degree, and cranberries and cherries withstand temperatures considerably below their freezing points without freezing. Gooseberries have been stored for three months at a temperature of 4° C. below their freezing point and only 11 percent of the fruit were frozen. In general, fruits and vegetables with waxy epidermal coverings may be undercooled much farther than plants in which the cutinized or suberized layer is not so pronounced; for instance, lettuce, celery, and cauliflower are very easily inoculated and will not undercool much below their freezing points. Other factors besides these waxy coverings apparently also influence the degree of undercooling, as plugs of potatoes with the cut surfaces exposed will undercool as much as do whole tubers. It is quite possible that the concentration of the cell sap has considerable to do with these phenomena. Wright and Taylor have shown that the rate at which potatoes are cooled influences the degree below their freezing points to which they can be cooled without freezing. To put it briefly, a very rapid or a very slow fall in the temperature is not favorable to low undercooling. Other factors as yet unrecognized are undoubtedly concerned in these phenomena.

A survey of the work on plant physiology, and especially on the phases relating to low-temperature storage and freezing, impresses one with the aptness of the statement frequently made, that "plants are water with some other things in it." This is especially applicable to fruits and vegetables which are commonly placed in cold storage. The water content of this type of produce varies from about 95 to 65 percent. The chemical processes in the plant are in a watery solution, and, as far as they have been investigated, follow much the same course as they do outside the plant. In freezing, the apple or potato behaves in about the same way as does a solution as regards undercooling, inoculation, and the crystallizing out of the water. We have, of course, in this aqueous solution a great many interrelated reactions going on at the same time, and knowledge of each process and of its relation to the other processes is necessary for a good understanding of the subject.

Bureau of Plant Industry,
U. S. Department of Agriculture

REVISION OF THE SOUTH AMERICAN SPECIES OF CUSCUTA

I

T. G. Yuncker

(Received for publication March 25, 1922)

In a recent study of the North American and West Indian species of Cuscuta[1] the writer had the opportunity of examining a number of specimens from South America. It was apparent that the species occurring in the two continents were closely related, and it was thought that a careful study should also be made of those of South America. The present paper presents the results of a study of those species known to occur in South America and the Galapagos Islands.

Ruiz and Pavon,[2] in 1798, described two species of Cuscuta which seem to have been the first to be listed from South America. Humboldt, Bonpland, and Kunth,[3] in 1818, described four species from the western coast. Choisy,[4] in 1841, in his monograph included several species from different parts of the continent, while Gay,[5] in 1849, listed five species as occurring in Chile. Engelmann,[6] in his monograph of the genus, described all the species then known and is the only writer who has given us anything like an adequate treatment of the group. Progel,[7] in 1871, described the species for Brazil, and Reiche,[8] in 1910, described eight species for Chile.

Thirty-eight species are included in this paper, seven of which are believed to be new to science. In addition to these there are five new varietal segregations which have seemed necessary for an adequate understanding of the species in which they occur. Of these thirty-eight species, eight are also found in North America or the West Indies, or in both. Only one species, *Cuscuta epithymum* Murr., belonging in the subgenus Succuta, which is typically an Old World group, has been seen from South America, and of this but one specimen. All the other species belong to the subgenus Grammica, which also includes all those of North America and the West Indies, with the exception of four introduced from Europe and of *Cuscuta*

---

[1] Yuncker. Illinois Biological Monographs 6: 95. 1921.

[2] Ruiz and Pavon. Fl. Peruv. et Chili. 1: 69. 1798.

[3] Humboldt, Bonpland, and Kunth. Nov. gen. et spec. pl. 3: 121. 1818.

[4] Choisy. Mém. Soc. Phys. Hist. Nat. Genève 9: 261. 1841.

[5] Gay. Histoire de Chile 4: 445. 1849.

[6] Engelmann. Trans. Acad. Sci. St. Louis 1: 453. 1859.

[7] Progel, in Martius' "Flora Brasiliensis" 7: 372. 1871.

[8] Reiche. Flore de Chile 5: 166. 1910.

exaltata Engelm., belonging in the subgenus Monogyna. The majority of the species belong in the section Eugrammica which is characterized by having a circumscissile capsule. None, however, possess capsules with as definite a line of circumscission as do some of the Mexican or Old World species, and a few which I consider as circumscissile have capsules that do not open easily until fully ripened. Some are found infesting trees, a characteristic exhibited by one or two Mexican species. Most, however, prefer herbaceous hosts.

Many points doubtful to Engelmann because of poor and inadequate specimens have been explained, it is believed, through the examination of more abundant materials. Several species have been moved out of the section in which Engelmann placed them into others because of this fact.

Excepting those species that also occur in North America or the West Indies, and which are illustrated in the paper[9] on those species, all are pictured, many of them, it is believed, for the first time. The sketches were all drawn to scale with the aid of an Abbé camera lucida. References to the principal papers and illustrations are given for each species.

During the progress of the study the writer had the privilege of examining a number of collections and was greatly aided by the courtesy shown him by the persons in charge of those collections. In all cases he was granted every consideration for the furtherance of the work and desires to take this opportunity of expressing his appreciation. Some of the collections were loaned for detailed study, and in other instances fragments of the specimens were generously supplied. The most valuable collection in this country is the Engelmann herbarium at the Missouri Botanical Garden, which includes Engelmann's types. In addition to this, the other collections studied are those belonging to the following institutions and individuals: Dr. Otto Buchtien, La Paz, Bolivia; Institute at Butantan, Brazil; Botanic Institute, Dahlem; Field Museum; Boissier Herbarium and the general herbarium of the Botanic Institute of the University of Geneva; Gray Herbarium; Royal Botanic Garden at Kew; New York Botanical Garden; Museum of Natural History, Trinidad, Paraguay; Museum of Natural History, Paris; Imperial Museum, Stockholm; United States National Herbarium; Botanical Museum, Utrecht; University of Zürich. In addition to these, I wish also to thank Professor and Mrs. E. W. D. Holway of the University of Minnesota and Dr. Ivar Holmgren of the Botanical Institute of the University of Stockholm, who collected and sent to the writer many excellent specimens of Cuscuta while on journeys in South America.

SYSTEMATIC ARRANGEMENT OF THE SPECIES

For the descriptions of the genus, subgenera, sections, and subsections, together with the synonymy for each, see Yuncker, Ill. Biol. Monogr. **6**: 110 *et seq.* 1921.

---

[9] Yuncker, *l.c.*

KEY TO THE SUBGENERA

Styles more or less united (no species belonging in this subgenus were found from South America) .... MONOGYNA.
Styles distinct
  Stigmas linear-elongated .... SUCCUTA.
  Stigmas capitate .... GRAMMICA.

Subgenus SUCCUTA (Des Moulins) Yuncker

1. *Cuscuta epithymum* Murray

For the description and synonomy of this species see Yuncker, Ill. Biol. Monogr. **6**: 112, figs. 2, 86, and 145. 1921.

Specimen examined: ARGENTINA: Prov. Buenos Ayres, on *Medicago sativa* (Osten *33*).

Subgenus GRAMMICA (Loureiro) Engelmann

KEY TO THE SECTIONS

Capsule circumscissile .... EUGRAMMICA.
Capsule remaining closed .... CLISTOGRAMMICA.

Section EUGRAMMICA Engelmann

KEY TO THE SUBSECTIONS

Styles more or less subulate, at least in fruit .... SUBULATAE.
Styles of about the same thickness throughout
  Flowers subtended by bracts .... LEPIDANCHOPSIS.
  Flowers not subtended by bracts
    Divisions of the perianth mostly obtuse .... OBTUSILOBAE.
    Divisions of the perianth mostly acute or acuminate .... LEPTILOBAE.

Subsection SUBULATAE Engelmann

KEY TO THE SPECIES

Calyx lobes broad, obtuse
  Flowers without infrastamineal scales .... 2. *C. grandiflora.*
  Flowers with definite infrastamineal scales
    Scales very short, not reaching to the middle of the tube .... 3. *C. brevisquamata.*
    Scales reaching to the middle of the tube or mostly longer
      Styles none, or extremely short
        Capsule papillate, styles very short but evident .... 4. *C. argentinana.*
        Capsule smooth, styles apparently none .... 5. *C. microstyla.*
      Styles nearly as long as the ovary, or mostly longer
        Anthers sessile, scales not reaching to the stamens .... 6. *C. chilensis.*
        Anthers not sessile, scales mostly reaching the stamens
          Flowers commonly 4–6 mm. long, styles mostly longer than the ovary
            Corolla lobes ovate, mostly as long as the corolla tube, becoming spreading or reflexed .... 7. *C. odorata.*
            Corolla lobes orbicular, much shorter than the corolla tube, upright, becoming connivent in fruit .... 8. *C. globiflora.*
          Flowers 2.5–3 mm. long, styles shorter than the ovary .... 9. *C. boliviana.*

Calyx lobes acute or acuminate
  Flowers 5–9 mm. long, corolla lobes shorter than the tube..... 10. *C. foetida.*
  Flowers 2–3 mm. long, corolla lobes as long as or longer than the tube
    Scales narrow, oblong, bridged low, flowers purplish-red.... 11. *C. acutiloba.*
    Scales ovate, bridged at about the middle, flowers not purplish-red.. 12. *C. xanthochortus.*

2. *Cuscuta grandiflora* Humboldt, Bonpland, & Kunth

(Pl. I, fig. 6, *A–D* [10])

Cuscuta grandiflora Humboldt, Bonpland, & Kunth, Nova gen. et spec. pl. **3**: 123 (97 in folio edition), t. 213. 1818.—Choisy, Mém. Soc. Phys. Hist. Nat. Genève **9**: 278. 1841; and in DeCandolle, Prodromus **9**: 457. 1845.—Engelmann, Trans. Acad. Sci. St. Louis **1**: 477. 1859. Not Wallich.

Stems medium. Flowers 4–6 mm. long, pentamerous, yellowish but commonly with a darker band of color about the throat of the corolla, on pedicels mostly shorter than, or, less frequently exceeding the length of the flowers, in few- to many-flowered, loose, cymose clusters; perianth segments finely fimbriate with short, filamentous processes; calyx broad, about as long as the corolla tube; lobes triangular-ovate, obtuse, not overlapping; corolla shallowly and broadly campanulate; lobes upright to spreading, broadly ovate, overlapping, obtuse; stamens attached below the sinuses, not reaching the middle of the corolla lobes; anthers oval, shorter than the subulate filaments; scales none; styles subulate, stout, shorter than the globose ovary; stigmas comparatively small, flattened or globose. Capsule depressed-globose, circumscissile, with the withered corolla at its base, all parts of the ovary and capsule papillate; seeds 3–4 in each capsule, rough, roundish, 1.5–2 mm. long, hilum oblong.

This is one of the most easily distinguished species because of the papillate fruit and the large flowers lacking scales.

Type locality: "Prope Santa Fe de Bogota," Colombia. *Distribution:* In the Andean region from central Colombia to northern Argentina.

Specimens examined: ARGENTINA: Prov. Catamarca, Dept. Andalgala (Jörgensen *1420*); Prov. Salta, Nevado del Castillo (Hieronymus and Lorentz *124*).

BOLIVIA: (Fries *1287*); La Paz (Bang *115*, in part; E. W. D. and Mary M. Holway, March 21, 1920, March 31, 1920; Buchtien *2946*, *4501*, and in 1912); between Palca and La Paz (Pflanz *40*, *454*); Sorata (Williams *2396;* Mandon *1479*, *1499;* E. W. D. and Mary M. Holway May 3, 1920; Weddell *4518*); Unduavi (Buchtien *460*, *752;* Rusby *2002*, *2003*); Pelechuco (Williams *2490*).

CHILE: (Edmonston).

COLOMBIA: (Humboldt, a fragment believed to be part of the type, in the Engelmann Herbarium; Voyage de J. Triana, 1851–57); Bogota (Holton *543;* Goudot).

ECUADOR: Latacunga (Holmgren *594*); Ambato (Pachano *140*).

PERU: (Gay); Dept. du Cuzco (Weddell *4768*).

3. **Cuscuta brevisquamata** n. sp.

(Pl. I, fig. 1, *A–E*)

Stems medium. Flowers 4–5 mm. long, on pedicels mostly longer than the flowers, in loose, few-flowered, umbellate or cymose clusters; calyx loose and spreading from the corolla; lobes triangular-ovate, obtuse, not overlapping; corolla campanulate, becoming urceolate in fruit; lobes shorter than the tube, ovate, obtuse, upright, edges finely serrate and uneven; stamens shorter than the lobes; anthers oval or oblong, about as long as the filaments; scales very short, not reaching to the middle of the tube, truncated or triangular, bridged at about the middle, with a few medium-length processes about the top, or sometimes these lacking; styles subulate, shorter than the depressed-globose ovary; stigmas comparatively large, peltate. Capsule depressed-globose, circumscissile, intrastylar aperture large, withered corolla about the upper part; seeds about 2.5 mm. long, reddish, usually four in each capsule, hilum short.

---

[10] The plates referred to will be found at the end of the second paper of this series.

This species bears a resemblance to *C. grandiflora* from which it differs in the possession of scales and a smooth capsule, and also to *C. globiflora* from which it differs by having pedicellate flowers, very short scales, and overlapping calyx lobes.

Type locality: "Sierra Achala de Córdoba, Argentina." *Distribution:* Known only from the type locality.

Specimens examined: ARGENTINA: Sierra Achala de Córdoba (Hieronymus Feb. 2, 1883, the type, in the Botanical Institute at Dahlem, a fragment in the author's herbarium).

4. **Cuscuta argentinana** n. sp.

(Pl. II, fig. 11, *A—E*)

Stems medium. Flowers 2–3 mm. long, on pedicels shorter than or exceeding the flowers, in loose, umbellate, or cymose clusters; calyx shallowly campanulate or rotate; lobes ovate or triangular, obtuse, about as long as the corolla tube; corolla shallowly campanulate-rotate; lobes spreading, ovate, obtuse, longer than the tube, edges more or less revolute and finely serrate; stamens inserted at the sinus, curving in over the ovary, filaments stout, subulate, and equal to or longer than the oval anthers; scales thick, exceeding the tube, spatulate, fringed with short processes, bridged very low; ovary depressed-globose, papillate; styles very short, stout; stigmas flattened. Capsule depressed-globose, papillate, circumscissile, with a thickened collar about the large intrastylar aperture; seeds two to four in each capsule, about 2 mm. long, oval, hilum oblique.

This species resembles *C. grandiflora* in many respects but differs from it in the possession of scales, shorter styles, stamens inserted at the sinus, and in not having fimbriate perianth segments.

Type locality: Argentina. *Distribution:* Known only from the type locality.

Specimen examined: ARGENTINA: (Jörgensen *1160*, the type, in the U. S. National Herbarium as sheet no. 704,804).

5. *Cuscuta microstyla* Engelmann

(Pl. I, fig. 4, *A–E*)

Cuscuta microstyla Engelmann, Trans. Acad. Sci. St. Louis 1: 506. 1859.—Progel in Martius, Flora Brasiliensis 7: 386. 1871.—Reiche, Fl. Chile 5: 170. 1910.

Stems medium. Flowers membranous, 3–4 mm. long, on pedicels shorter than or exceeding the length of the flowers, in lateral, cymose, few-flowered clusters; calyx lobes as long as the corolla tube, ovate, obtuse, slightly or not at all overlapping; corolla shallow, campanulate; lobes upright to spreading, about equaling or longer than the tube, ovate, obtuse, slightly overlapping at the base; stamens shorter than the lobes, subulate filaments longer than the oval anthers; scales reaching the stamens, broadly spatulate, sometimes more or less truncated, fringed with short processes about the upper part, bridged below the middle; ovary globose-conic; styles apparently lacking; stigmas sessile, more or less peltate and frilled. Capsule globose or slightly conic, circumscissile, the withered corolla about it and towards the base; seed about 1.5 mm. long, ovate, hilum linear and at the end.

The fragment of the type examined was so small and under-developed that it was impossible to distinguish clearly all the characters. Engelmann states with his original description that "the only specimen seen is very young with only a few flowers open, and a single half-grown capsule," which may account for the fact that he considered the capsule not circumscissile. The specimen collected by Fries, however, was well developed and abundant and seems to be this species. It showed definite characteristics, as described, including a definitely circumscissile capsule. My description, therefore, is drawn mainly from Fries' collection and assumes that this is probably the same species as Reynolds' collection.

Type locality: Chile, on the volcano of Antuco. *Distribution:* Central Chile and northern Argentina.

Specimens examined: ARGENTINA: Prov. Jujuy, Nevado de Chañi (Fries *906*).

CHILE: Volcano Antuco (Reynolds *95*, the type, in the herbarium of the Royal Botanic Gardens, Kew).

6. *Cuscuta chilensis* Ker-Gawler

(Pl. II, fig. 10, *A–E*)

Cuscuta chilensis Ker-Gawler, Bot. Reg. **7**, Pl. 603. 1821.—Choisy, Mém. Soc. Phys. Hist Nat. Genève **9**: 275. 1841; and in De Candolle, Prodromus **9**: 455. 1845.—Gay, Hist. de Chile **4**: 446. 1849.—Engelmann, Trans. Acad. Sci. St. Louis **1**: 478. 1859. —Reiche, Fl. Chile **5**: 168. 1910. Not Bert. mss. ex Choisy, nor Hort. Frib. ex Engelmann.

Cuscuta odorata Poeppig ex Choisy in De Candolle, Prodromus **9**: 455. 1845.

Cuscuta aurea Philippi, Ann. Univ. Chile **90**: 224. 1895.

Stems medium. Flowers 5–7 mm. long, smooth or infrequently slightly papillate, subsessile to sessile, in dense lateral clusters; calyx much shorter than the corolla tube, lobes ovate, infrequently unequal, obtuse, slightly overlapping; corolla cylindrical, lobes triangular-ovate or infrequently oblong-ovate, obtuse or rarely acutish, much shorter than the corolla tube; anthers linear-oblong, comparatively large, frequently purple, subsessile or sessile; scales shorter than the tube, bridged at about the middle, profusely fringed with medium-length processes; styles subulate, about equal to the globose-conic ovary; stigmas large and frequently more or less convoluted and reddish. Capsule circumscissile, globose or depressed-globose or appearing conical because of the subulate styles, with the withered corolla about it; seeds two to four in each capsule, oval, 1.5–2 mm. long, hilum short.

This species is closely allied with *C. odorata* and is frequently confounded with it, but differs from that species in the possession of a longer corolla with comparatively shorter lobes, and large, subsessile or sessile anthers.

Type locality: Valparaiso (?), Chile. *Distribution:* Principally in central and northern Chile, two specimens from Argentina, one from Peru, and one from Brazil having been seen. The specimens found outside of Chile appear typical in all respects. A penciled notation on the Brazilian specimen in the herbarium at Kew, apparently by Engelmann, expresses doubt as to that specimen's being from Brazil.

Specimens examined: ARGENTINA: (Lorentz *214*); Prov. Mendoza (Smith in 1890–91).

BRAZIL: (Sellow).

CHILE: (Gay *817;* another collection without number or date; Edmonston; Gillies; Savatier *1751;* Maximowicz; Poeppig); Concepcion (Mertens); San Cristobel (Hastings *146, 291, 395*); Santiago (Ball in 1882; Philippi *656*); Zapallar (E. W. D. and Mary M. Holway Jan. 30, 1920); Linares (E. W. D. and Mary M. Holway Dec. 24, 1919); Atacama (Morong *1143*); Valparaiso (Rusby *2001;* Wilkes; Cuming *350;* Andersson in 1852; Harvey in 1856; Buchtien *4502, 4503;* Lechler *1501*); Angol (Kuntze in 1892); Talca (Philippi in 1888); Panamavida (E. W. D. and Mary M. Holway Dec. 24, 1919); Uspallala Pass (Buchtien *1157, 4507,* Jan. 16, 1903); Volcano Antuco (Husbands *1015*); Lota (Lee in 1888).

PERU: (Martinet).

7. *Cuscuta odorata* Ruiz & Pavon

Cuscuta odorata Ruiz & Pavon, Fl. Peruv. **1**: 69, Pl. 105, fig. *a.* 1798.—Engelmann, Trans. Acad. Sci. St. Louis **1**: 477. 1859.—Reiche, Fl. Chile **5**: 168. 1910. Not Choisy nor Poeppig.

Cuscuta intermedia Choisy, Mém. Soc. Phys. Hist. Nat. Genève **9**: 275, Pl. 2, fig. 3. 1841; and in De Candolle, Prodromus **9**: 445. 1845.—Gay, Hist. de Chile **4**: 447. 1849.

Cuscuta purpurata Philippi, Ann. Univ. Chile **90**: 225. 1895.

Cuscuta fragrans Rusby, Mem. Torr. Bot. Club **6**: 85. 1896.

Stems medium. Flowers 4–6 mm. long, and nearly as wide in some specimens, subsessile in dense, lateral clusters; calyx shorter than, or mostly about as long as, the corolla tube; lobes ovate-orbicular, frequently unequal, obtuse, overlapping; corolla campanulate; lobes upright or spreading and in some flowers reflexed, about as long as the tube in most specimens, rarely about half as long, ovate, obtuse, overlapping; stamens reaching to about the middle of the corolla lobes, or shorter, filaments stout, subulate, and longer or shorter than the oval anthers; scales large and prominent, about reaching the stamens or, rarely, shorter, densely fringed with medium to short processes, bridged at the middle or below; styles subulate, about as long as, or shorter than, the depressed-globose ovary. Capsule depressed-globose, circumscissile, carrying the withered corolla about it; seeds about 2 mm. long, hilum linear.

The length of the corolla lobes in comparison with the tube varies considerably with the different specimens, as do also the shape of the scales and the length of the styles.

KEY TO THE VARIETIES

Corolla lobes about as long as the tube .... *typica.*

Corolla lobes half to two thirds as long as the tube

Scales narrow, oblong or triangular . . . . *Holwayana.*
Scales ovate or oval, not particularly narrow, capsule comparatively large, styles short . . . . *botryoides.*

Cuscuta odorata typica[11]

(Pl. IV, fig. 21, *A–E*)

Corolla lobes about as long as the tube, styles about as long as, or exceeding, the length of the ovary, scales mostly oval.

Type locality: Peru. *Distribution:* Ecuador, Peru, northern Chile, and Bolivia.

Specimens examined: Bolivia: Sorata (Bang *1303*, the type of *C. fragrans;* Mandon *1480*, mixed with *C. globiflora* in some collections).

Brazil: Minas Geraes (St. Hilaire *D. 482*. This specimen has more ovate calyx lobes and shorter corolla lobes).

Chile: Coquimbo (Gay *38*).

Ecuador: Loja (Seeman *852;* Rose, Pachano, and Rose *23272*); Huigra (Rose and Rose *22256;* E. W. D. and Mary M. Holway Aug. 3, 1920).

Peru: (Martinet *1027;* another collection without number or date; Gay *2168;* Herb. Ruiz., taken to represent the type, a fragment in the Engelmann herbarium; Herb. Pavon.; Matthew *486;* Savatier *460*, *1362;* Capt. Wilkes); Lima (Nation in 1862); Carabaya (Weddell *4693*).

Cuscuta odorata Holwayana n. var.

(Pl. IV, fig. 21, *F*)

Corolla lobes half to two thirds as long as the tube, scales narrow, oblong or triangular, more sparingly fringed than in the typical variety, capsule not so depressed.

Type locality: Sorata, Bolivia. *Distribution:* Known only from the type locality.

Specimens examined: Argentina: Prov. Jujuy (Claren *11692*).

Bolivia: Sorata (E. W. D. and Mary M. Holway April 12, 1920, the type, a fragment in the author's herbarium).

Cuscuta odorata botryoides Engelmann

(Pl. IV, fig. 21, *G*)

Cuscuta odorata botryoides Engelmann, Trans. Acad. Sci. St. Louis 1: 477. 1859.

Corolla lobes about half as long as the tube, calyx lobes uneven, capsule large, depressed-globose, styles short, intrastylar aperture large and gaping.

Type locality: Southern Brazil. *Distribution:* Known only from the type locality.

Specimens examined: Southern Brazil (Lobb *49*, the type, in the herbarium of the Royal Botanic Gardens, Kew, a fragment in the Engelmann herbarium).

8. *Cuscuta globiflora* Engelmann

(Pl. II, fig. 9, *A–E*)

Cuscuta globiflora Engelmann, Trans. Acad. Sci. St. Louis 1: 520. 1859.

---

[11] It has seemed advisable, for a clear understanding of those species exhibiting varietal segregations, to designate the typical form or variety as variety *typica*. Where this term is used, it is meant to refer only to the normal or typical form for the species.

Stems medium. Flowers 4–5 mm. long and nearly as broad, texture thick and fleshy, sessile in few- to many-flowered, globular clusters, each flower frequently subtended by an ovate-orbicular bract, perianth segments commonly finely fimbriate with delicate filamentous processes; calyx deep, reaching nearly to the corolla lobes; lobes orbicular, overlapping; corolla urceolate; lobes ovate-orbicular, overlapping, about half as long as the tube, upright, becoming connivent in fruit; stamens short, filaments stout and shorter than the oval anthers; scales large, reaching the stamens, bridged at about the middle or below, fringed with short or medium-length processes; styles subulate, about equal to the globose, depressed ovary; stigmas elongated-globose or globose. Capsule depressed-globose, circumscissile, with the withered corolla about it; seeds about 1.5 mm. long, two to four in each capsule, seeds in some capsules red, hilum short, oblong.

Type locality: "Cuzco, Bolivia." *Distribution:* In western Bolivia and northern Argentina.

Specimens examined: ARGENTINA: Prov. de Catamarca, Dept. de Andalgala (Jörgensen *1702*).

BOLIVIA: (Buchtien *113*); Mt. (?) Cuzco (Pentland, the type, a fragment in the Engelmann herbarium); La Paz (Buchtien *753*, *3235;* E. W. D. and Mary M. Holway Mar. 25, 1920; Bang *115* in part; Rusby *2004*); between Palca and La Paz (Pflanz *382*); Sorata (Mandon *1480*, mixed with *C. odorata* in some collections; Bang *1304*); Cotaña (Buchtien *133*, *4504*).

9. **Cuscuta boliviana** n. sp.

(Pl. I, fig. 3, *A–E*)

Stems medium to slender. Flowers 2.5–3 mm. long, subsessile or sessile in dense, few-flowered, globular clusters; calyx nearly as long as the corolla tube; lobes ovate-orbicular, obtuse, overlapping, infrequently some lobes strongly carinate, edges slightly uneven but not fimbriate; corolla campanulate, soon becoming somewhat globular about the developing capsule; lobes upright or spreading, ovate, obtuse, shorter than the tube; scales reaching the stamens, oblong, truncated, moderately fringed with medium-length processes particularly about the top, bridged below the middle; anthers about equaling or shorter than the stoutish filaments; ovary globose, slightly thickened about the style bases; styles stout and slightly subulate, shorter than the ovary; stigmas globose. Capsule depressed-globose, circumscissile, with the withered corolla about it but not covering the partly exposed capsule; seeds about 1.5 mm. long, ordinarily four in each capsule, reddish in the specimen examined, hilum short, oblong, oblique.

This species is closely related to *C. globiflora* but differs from it in having smaller flowers, spreading corolla lobes, partially exposed capsule, and shorter styles. Also the perianth segments are not fimbriate as in that species. It differs from *C. cristata* in the possession of a circumscissile capsule.

Type locality: Padcayo, Bolivia. *Distribution:* Known only from the type locality.

Specimens examined: BOLIVIA: Padcayo (Fiebrig *2523*, the type, in the Buchtien herbarium, a fragment in the author's herbarium).

10. *Cuscuta foetida* Humboldt, Bonpland, & Kunth

Cuscuta foetida Humboldt, Bonpland, & Kunth, Nova gen. et sp. pl. 3: 122 (96 in folio edition). 1818.—Choisy, Mém. Soc. Phys. Hist. Nat. Genève 9: 285. 1841; and in

De Candolle, Prodromus 9: 460. 1845.—Engelmann, Trans. Acad. Sci. St. Louis 1: 478. 1859. Not Hook. & Arnot, Bot. Beechy ex Engelmann, *l.c.*

Cuscuta corymbosa Jussieu in herb. ex Engelmann, Trans. Acad. Sci. St. Louis 1: 478. 1859.

Stems medium to coarse. Flowers 5–9 mm. long, sessile in dense, many-flowered, globular clusters, sometimes nearly an inch in diameter; calyx nearly as long as the corolla tube or infrequently only half as long as the tube; lobes triangular-ovate, acute to acuminate, overlapping; corolla cylindrical, becoming somewhat urceolate about the developing capsule, slightly saccate between the stamen attachments toward the base; lobes triangular-ovate, acute to acuminate, smooth or slightly papillate, overlapping, upright to spreading or becoming reflexed in fruit, about half or three quarters as long as the tube; stamens much shorter than the lobes, oval-oblong; anthers subsessile or on short, stout filaments; scales oblong or somewhat oval, shorter than the tube, fringed with short processes, bridged at about a quarter of their height; styles about equal to the subglobose ovary, subulate and tapering into the ovary. Capsule globose, slightly depressed, circumscissile, with the withered corolla about it; seeds about 1.5 mm. long, somewhat rostrate, hilum short, oblong.

Cuscuta foetida typica

(Pl. V, fig. 24, *A–E*)

Flowers 5–7 mm. long. Calyx nearly as long as or equaling the corolla tube; calyx and corolla lobes broadly triangular-ovate.

Type locality: Quito, Ecuador. *Distribution:* In the Ecuadorian Andes.

Specimens examined: ECUADOR: (Seeman; Jameson in 1864); Quito (Humboldt, a fragment taken to be part of the type in the Engelmann herbarium; Couthouy in 1855; Spruce *5017;* Holmgren *442;* E. W. D. and Mary M. Holway Aug. 15, 1920); Cuenca (E. W. D. and Mary M. Holway Sept. 10, 1920).

Cuscuta foetida pycnantha (Bentham) n. comb.

(Pl. V, fig. 24, *F*)

Cuscuta pycnantha Bentham, Pl. Hartw. p. 226. 1839.

Flowers 7–9 mm. long, corolla slenderly cylindrical. Calyx much shorter than the corolla tube, calyx and corolla lobes narrower than in typical variety, scales oblong.

Type locality: Cuenca, Ecuador. (A fragment of the collection on which this variety is based, in the Engelmann herbarium, is labeled as from Colombia, and Engelmann so considered it in his citation in his monograph. However, a specimen of the same collector and number in the herbarium of the Royal Botanic Gardens at Kew is labeled as from near Cuenca, Ecuador, and a specimen of the same number in the Boissier herbarium gives similar information. All three specimens seem identical, and, inasmuch as no other specimens have been seen from Colombia, it is probable that an error occurred in labeling the specimen in the Engelmann herbarium.) *Distribution:* Known only from the type locality.

Specimens examined: ECUADOR: Cuenca (Hartweg *1238,* the type, in the Royal Botanic Gardens, Kew, a fragment in the author's herbarium).

11. *Cuscuta acutiloba* Engelmann

(Pl. I, fig. 5, *A–E*)

Cuscuta acutiloba Engelmann, Trans. Acad. Sci. St. Louis **1**: 478. 1859.

Stems medium to slender. Flowers 2.5–3 mm. long, on pedicels shorter than or exceeding the length of the flowers, in cymose clusters; calyx as long as the corolla tube; lobes overlapping and somewhat angled at the sinuses, triangular-ovate, acuminate or sometimes cuspidate; corolla purplish-red, campanulate, becoming urceolate and globular about the ripening capsule; lobes lanceolate, acute, reflexed, overlapping at the base, mostly longer than the tube; stamens much shorter than the lobes, orbicular anthers about equal to the filaments; scales oblong, narrow, reaching the stamens, sparingly fringed with short processes, bridged much below the middle; styles subulate, shorter than, and tapering into, the globose ovary. Capsule depressed-globose, membranous, early circumscissile, with the withered corolla about it; seeds 1–1.5 mm. long, roundish or ovate, ordinarily four in each capsule, hilum short.

This species is easily distinguished by the purplish-red color of the corolla, which is in marked contrast with the straw color of most of the other South American species.

Type locality: "At the bridge of Obragilla, Peru." *Distribution:* Bolivia and Peru.

Specimens examined: BOLIVIA: Sorata (Mandon *1481*).

PERU: Bridge of Obragilla (Mathews in 1857, the type, in the Royal Botanic Gardens, Kew).

12. *Cuscuta xanthochortus* Engelmann

Cuscuta xanthochortus Engelmann, Trans. Acad. Sci. St. Louis **1**: 486. 1859.—Progel in Martius, Flora Brasiliensis **7**: 379, Pl. 126, fig. 5. 1871.

Cuscuta corniculata racemulosa Engelmann, Trans. Acad. Sci. St. Louis **1**: 504. 1859.—Progel in Martius, Flora Brasiliensis **7**: 382, Pl. 127, fig. 3. 1871.

Stems slender. Flowers 2–3 mm. long, subsessile, or on pedicels shorter than the flowers, in racemose or cymose clusters; calyx shorter than or exceeding the length of the corolla; lobes triangular-ovate or somewhat lanceolate, acute, overlapping at the base, thickened at the point, sometimes spreading or even recurving; corolla shallowly campanulate; lobes longer than the tube, reflexed, triangular-ovate or lanceolate, acute, tips commonly inflexed, smooth, or somewhat papillate and fleshy; stamens shorter than the lobes, the oblong-oval anthers shorter than or equaling the subulate filaments; scales reaching the stamens or infrequently shorter, bridged at about the middle, fringed with short processes; thick and slightly subulate styles longer than or about equaling the globose ovary which is thickened at the top in the form of a collar about the style bases. Capsule globose, circumscissile, carrying the withered corolla about it. Mature seeds not seen.

Cuscuta xanthochortus typica

(Pl. III, fig. 18, *A–E*)

Flowers about 2 mm. long, on short pedicels in dense clusters; calyx lobes triangular, acutish, scarcely as long as the corolla tube.

Engelmann did not consider this as having a circumscissile capsule, but it is evidently so closely related to the following variety which has a definitely circumscissile capsule that, even though mature fruit was not seen, it is placed here.

Type locality: Porto Alegre, Brazil. *Distribution:* Southern Brazil and Argentina.

Specimens examined: ARGENTINA: (Niederlein *1308*).

BRAZIL: Porto Alegre (Father Joannes de Santa Barbara, the type of *C. xanthochortus*, a fragment in the Engelmann herbarium); southern Brazil (Sellow *2489*, taken to represent the type of *C. corniculata racemulosa*, a fragment in the Engelmann herbarium). Rio Grande do Sul (Malme *1002*, *1416*—these approach the next variety).

Cuscuta xanthochortus lanceolata n. var.

(Pl. III, fig. 18, *F*, *G*)

Flowers about 3 mm. long, subsessile in dense clusters. Calyx and corolla lobes ovate-lanceolate, acute.

Type locality: Maracayú Mountains, Paraguay. *Distribution:* Known only from Paraguay.

Specimens examined: PARAGUAY: (Balansa *2064*, *2066;* Anisits *462;* Page in 1854); Maracayú Mountains (Hassler *5113*, the type, in the herbarium of the Botanical Institute at Dahlem, a fragment in the author's herbarium, *4694*); between the rivers Apa and Aquidaban (Fiebrig); Asuncion (Fiebrig *6705*).

Subsection LEPIDANCHOPSIS Yuncker

Flowers sessile in compact, more or less continuous clusters, subtended by bracts; calyx lobes distinct or nearly so, obtuse or acute.

KEY TO THE SPECIES

Scales reaching the stamens, profusely fringed with long processes, corolla lobes about equal to the corolla tube
  Floral parts mostly obtuse or slightly acutish or cuspidate, bracts few, stigmas globose .... 13. *C. goyaziana.*
  Floral parts mostly acute, bracts more numerous, stigmas oval .... 14. *C. bracteata.*
Scales mostly not reaching the stamens, moderately fringed with medium-length processes, corolla lobes shorter than the tube .... 15. *C. serrata.*

13. **Cuscuta goyaziana** n. sp.

(Pl. III, fig. 13, *A–E*)

Stems medium. Flowers 4–5 mm. long, yellowish-orange in color, somewhat fleshy and thick in texture, with yellowish, pellucid, glandular-appearing cells, sessile, surrounded by one to three ovate, obtuse bracts. The type of inflorescence is not known, for the specimens examined were represented only by parts of inflorescences. Calyx as long as the corolla, sepals distinct or nearly so, ovate, obtuse, overlapping; corolla cylindrical or somewhat campanulate; lobes ovate, obtuse or slightly acutish, about as long as, or somewhat shorter than, the tube, overlapping, spreading; stamens shorter than the lobes, filaments about as long as the large, oval anthers; scales prominent, exserted, bridged at about the middle, profusely fringed with long processes; styles slender, much longer than the globose ovary; stigmas globose. Capsule globose, circumscissile, with a thickened collar about the intrastylar aperture; seeds about 2 mm. long, ordinarily four in each capsule, oval or ovate, hilum short, linear, perpendicular.

The number of bracts present in the flowers of this species varies considerably with the different flowers. It is possible that with more abundant material some flowers might be found lacking them entirely. This species appears to represent a transition stage between those species not having bracts and those possessing them. It differs from *C. bracteata* in the shapes of the stigmas and of the floral parts.

Type locality: Prov. Goyaz, Brazil. *Distribution:* Known only from the type locality.

Specimens examined: BRAZIL: Prov. Goyaz (Glaziou *21810*, the type, in the herbarium of the Botanical Institute at Dahlem, a fragment in the author's herbarium).

14. *Cuscuta bracteata* Engelmann

(Pl. III, fig. 16, *A–E*)

Cuscuta bracteata Engelmann, Trans. Acad. Sci. St. Louis 1: 509. 1859.

Stems medium or coarse. Flowers reddish, 5–6 mm. long, sessile or subsessile, in loose spicate or paniculate clusters, subtended by two to five ovate, obtusish or acute, sometimes cuspidate, bracts; flower parts serrulate and with numerous pellucid, glandular-appearing cells; sepals distinct, ovate, acute, sometimes cuspidate, as long as the corolla tube; corolla subcylindrical, lobes upright or spreading, ovate-lanceolate, acute, not quite as long as the tube; stamens shorter than the corolla lobes, filaments nearly equal to the oval to oblong anthers; scales reaching the stamens, abundantly fringed with long processes, bridged at about the middle; styles not subulate, longer than the globose ovary; stigmas oval-elongated. Capsule globose-ovoid, circumscissile, membranaceous, carrying the withered corolla at the apex; seeds 2–2.5 mm. long, 2–4 in each capsule, ovoid, hilum short, longitudinal.

This species has a definitely circumscissile capsule, although Engelmann, because of the undeveloped condition of the type specimen, did not so consider it.

Type locality: Prov. Goyaz, Brazil. *Distribution:* Known only from the type locality.

Specimens examined: BRAZIL: Prov. Goyaz (Gardner *3348*, the type, in the Royal Botanic Gardens, Kew. A fragment in the Engelmann herbarium).

15. **Cuscuta serrata** n. sp.

(Pl. III, fig. 17, *A–E*)

Stems slender or medium, disappearing early from between the floral masses. Flowers about 5 mm. long, yellowish or reddish, sessile, in dense, compact, globular or elongated, closely adherent clusters, surrounded by many (5–15) ovate-lanceolate, acute or acuminate bracts, all parts of the flowers serrate and having many pellucid, glandular-appearing cells; sepals distinct, ovate-lanceolate, acute or acuminate, sometimes cuspidate, as long as the corolla tube, more or less squarrose, bract and sepal tips sometimes recurving; corolla cylindrical; lobes ovate, acute to acuminate, sometimes cuspidate, overlapping, shorter than the tube, upright or spreading; stamens shorter than the lobes; filaments shorter than or equaling the oval-oblong anthers; scales mostly not reaching the stamens, bridged at or slightly above the middle, fringed with medium-length processes; styles slender, longer than the globose-ovoid ovary; stigmas globose. Capsule globose-ovoid, circumscissile, carrying the withered corolla at the apex; seeds 1–1.25 mm. long, roundish, hilum linear.

This species differs from *C. bracteata* with which it is closely allied in the shapes of the stigmas, bracts, sepals, and scales and in the inflorescence. From *C. glomerata,* a prairie species of North America, it differs mainly in the possession of a circumscissile capsule.

Type locality: Prov. Goyaz, Brazil. *Distribution:* Provinces of Goyaz and São Paulo, Brazil.

Specimens examined: BRAZIL: Prov. Goyaz (Glaziou *21811,* the type, in the Botanical Institute at Dahlem, a fragment in the author's herbarium, *21811½*; Ule *3009*); Prov. São Paulo (Saint Hilaire C^1 *711*).

Subsection OBTUSILOBAE Engelmann

KEY TO THE SPECIES

Flowers about as broad as long, corolla lobes mostly as long as the tube

Corolla lobes more or less fleshy, papillate, acute tips inflexed, styles mostly shorter than the ovary........................ 16. *C. corniculata.*

Corolla lobes not fleshy, or, if so, not papillate, obtuse, tips mostly not inflexed, styles equaling or exceeding the ovary

Calyx lobes slightly or not at all overlapping, scales reaching the stamens... 17. *C. incurvata.*

Calyx lobes distinctly overlapping, scales scarcely reaching the stamens.. 18. *C. trichostyla.*

Flowers elongated, corolla lobes much shorter than the cylindrical tube of the corolla

Calyx divided to the middle or below, lobes orbicular............ 19. *C. orbiculata.*

Calyx lobes shorter than the calyx tube

Calyx lobes obtuse

Flowers mostly about 3 mm. long, scales about reaching the filaments, bridged at the middle or above.............. 20. *C. americana.*

Flowers larger, scales shorter than the tube, bridged below the middle.. 21. *C. corymbosa.*

Calyx lobes acute.. 22. *C. prismatica.*

16. *Cuscuta corniculata* Engelmann

(Pl. II, fig. 12, *A–E*)

Cuscuta corniculata Engelmann, Trans. Acad. Sci. St. Louis 1: 504. 1859.

Cuscuta corniculata sphaerocyma Engelmann, Trans. Acad. Sci. St. Louis 1: 504. 1859. —Progel in Martius, Flora Brasiliensis 7: 382, Pl. 127, fig. 4. 1871.

Stems slender to medium. Flowers about 3 mm. long, subsessile in dense lateral clusters; calyx shorter than the corolla tube; lobes ovate, obtusish, slightly, or not at all, overlapping; corolla campanulate, slightly fleshy; lobes ovate, acute, spreading, about as long as, or shorter than, the tube, tips inflexed and with the cells slightly papillate; stamens slightly shorter than the lobes, the oval anthers shorter than the stout, subulate filaments; scales reaching the stamens, abundantly fringed with medium-length processes, bridged at about the middle; styles stout and but slightly subulate, shorter than or nearly equaling the globose ovary which is thickened in the form of a ring about the wide, gaping, intrastylar aperture. Capsule globose, exserted, irregularly circumscissile in a zone of very thin tissue toward the base, the withered corolla towards its base; seeds one to four in each capsule, 1.5–2 mm. long, ovate, hilum perpendicular.

Engelmann did not consider the capsule of this species as being circumscissile. However, fully matured capsules break away easily in an irregular, but definite, line of cleavage. The thickened top and wide intrastylar aperture distinguish this species.

Type locality: Prov. Goyaz, Brazil. *Distribution:* Prov. Goyaz, Brazil, Venezuela and Colombia.

Specimens examined: Colombia: Villavicencio (Pennell *1453*); Prov. Bogota (Triana in 1851–57).

Venezuela: Rio Meta (Karsten, taken to represent a co-type, a fragment in the Engelmann herbarium).

17. *Cuscuta incurvata* Progel

(Pl. II, fig. 7, *A–E*)

Cuscuta incurvata Progel in Martius, Flora Brasiliensis **7**: 379, Pl. 126, fig. 4. 1871.
Cuscuta incurvata apaensis Chodat & Hassler, Bull. Herb. Boissier II, **7**: 280. 1907.

Stems medium to slender. Flowers about 2 mm. long, subsessile or short-pedicellate, in dense cymose or umbellate clusters; calyx mostly shorter than the corolla tube; lobes ovate, obtuse, scarcely imbricate; corolla widely campanulate; lobes nearly as long as the corolla tube, reflexed or upright, ovate, obtuse or slightly acutish; stamens shorter than the corolla lobes; oval anthers shorter than or about equaling the stout, subulate filaments; scales reaching the stamens, fringed with short processes, bridged below the middle; stout and scarcely, if at all, subulate styles as long as, or longer than, the globose ovary. Capsule depressed-globose, membranaceous about the base, becoming late and irregularly circumscissile, carrying the withered corolla about it; seeds ovate-oblong, four in each capsule, about 1.5 mm. long, hilum oblique-perpendicular.

This species differs from *C. trichostyla*, to which it bears some resemblance, in the fact that the calyx lobes do not broadly overlap and by the possession of longer scales.

Type locality: "Prope Lagoa Santa." *Distribution:* The only specimens seen by the writer were from Paraguay.

Specimens examined: Paraguay (Fiebrig in 1909; Anisits *2395*, *2555*, *2854*); between the rivers Apa and Aquidaban (Fiebrig *4254*, *5083*); upper course of the river Apa (Hassler *8178*, the type number of variety *apaensis*).

18. *Cuscuta trichostyla* Engelmann

Cuscuta trichostyla Engelmann, Trans. Acad. Sci. St. Louis **1**: 495. 1859.—Progel in Martius, Flora Brasiliensis **7**: 383, Pl. 127, fig. 6. 1871.

Stems slender. Flowers 2.5–4 mm. long, subsessile in compact globular clusters; calyx not as long as, or about equaling, the corolla tube; lobes ovate-orbicular, obtuse, broadly imbricated, somewhat carinate towards the apex; corolla campanulate or slightly cylindrical, lobes shorter than the tube, ovate, obtuse, spreading or reflexed; stamens shorter than the lobes; filaments stoutish and subulate, about equal to or longer than the ovate anthers; scales not reaching the stamens, bridged below or at about the middle, sparingly fringed with medium-length processes, well attached; styles not subulate, or at the most only slightly so, much longer than the depressed-globose ovary. Capsule late and somewhat irregularly circumscissile, with the withered corolla about it, intrastylar aperture widely gaping; mature seeds not seen.

Engelmann did not consider the capsule of this species as opening, but in the specimens examined I find that it is late and irregularly, but definitely, circumscissile.

Cuscuta trichostyla typica

(Pl. III, fig. 15, *A–E*)

Corolla cylindric-campanulate, calyx not as long as the corolla tube, scales oblong, bridged below the middle.

Type locality: Panama. *Distribution:* Panama and Province of Para, Brazil.

Specimens examined: BRAZIL: Prov. Para, Santarem (Spruce *854*).

PANAMA (Tweedie, the type, a fragment in the Engelmann herbarium).

Cuscuta trichostyla carinata n. var.

(Pl. III, fig. 15, *F*, *G*)

Corolla campanulate, calyx about as long as the corolla tube, carinate toward the tips; scales ovate, bridged at about the middle.

Type locality: Paraguay. *Distribution:* Known only from the type locality.

Specimen examined: PARAGUAY (Fiebrig *487*).

19. **Cuscuta orbiculata** n. sp.

(Pl. IV, fig. 19, *A–E*)

Stems thick. Flowers 4–5 mm. long, subsessile in racemose-spicate clusters; calyx deep, nearly as long as the corolla tube; lobes orbicular, thick and fleshy, overlapping, edges finely irregular; corolla cylindrical, soon becoming urceolate because of the developing capsule; lobes short, ovate-orbicular, obtuse, overlapping, spreading to reflexed; stamens shorter than the lobes; filaments stout, equal to or shorter than the ovate anthers; scales about reaching the stamens, prominent, heavily fringed, bridged below the middle; styles slender, equal to or longer than the globose ovary; stigmas rather large. Capsule globose, circumscissile, the withered corolla enveloping it; only one seed found in each of the few capsules opened, orbicular, about 1.5 mm. long, hilum perpendicular.

This species resembles *C. globiflora* but differs in the type of inflorescence and in the shape of the calyx lobes. It differs from *C. americana* in the shapes of the calyx, scales, and capsule.

Type locality: Goyaz, Brazil. *Distribution:* Goyaz, Brazil, and the island of Fernando de Noronha.

Specimens examined: BRAZIL: Goyaz (Glaziou *21809*, the type, in the Royal Botanic Gardens, Kew, a fragment in the author's herbarium); Island of Fernando de Noronha (Moseley in 1873).

20. *Cuscuta americana* Linnaeus

For the description and synonymy of this species see Yuncker, Ill. Biol. Monogr. **6**: 122, figs. 22, 109, and 138. 1921.

Progel in Martius, Flora Brasiliensis **7**: 376. 1871, apparently considered this species as being made up of three varieties. I do not believe, however, that three varieties can be maintained, and, even with the two that are segregated, it is sometimes difficult to keep them distinct. The variety *congesta* as here considered includes both the typical variety and the variety *congesta* as proposed by Progel.

Cuscuta americana congesta Progel

Specimens examined: BRAZIL: (Gardner *1775*); Bahia (Salzman in 1845; Blanchet *736*); Goyaz, Buixas (Weddell *2208*); Minas Geraes (Claussen *306*); Rives de l'Araguay (Weddell *2298*); Matto Grosso, Cuyabá (Malme *1898*); Fernando de Noronha, Portuguese Bay (Ridley, Lea, and Ramage *73*); Rat Island (Moseley in 1873).

COLOMBIA: Santa Marta (Smith *1590*, *2549*); Barranquilla, Medellin, and Antioquia (Mayor *239*); Giradot (Rusby and Pennell *93*).

ECUADOR: Bodegas (Sodiro *113/9*).

VENEZUELA: (Karsten); Near Colonian Tovar (Fendler *2069*).

Cuscuta americana spectabilis Progel

Specimen examined: ARGENTINA: Prov. Jujuy (Fries *229*).

21. *Cuscuta corymbosa* Ruiz & Pavon

For the description and synonymy of this species see Yuncker, Ill. Biol. Monogr. **6**: 124, figs. 24, 104, and 110. 1921.

KEY TO THE VARIETIES

Flowers 4–8 mm. long, scales definite
  Flowers 6–8 mm. long, anthers subsessile, calyx usually exceeding the middle of the corolla tube . . . . *grandiflora.*
  Flowers 4–5 mm. long, filaments longer, calyx short, not reaching the middle of the corolla tube (no specimens of this variety have been seen from South America) . . . . *stylosa.*
Flowers about 4 mm. long, scales indefinite and thin . . . . *microlepis.*

Cuscuta corymbosa grandiflora Engelmann

Specimens examined: COLOMBIA: (Humboldt, taken to represent the type of this variety, a fragment in the Engelmann herbarium; Hartweg *1237*).

ECUADOR: Between Guaranda and Bodegas (Remy); Cotacachi (Holmgren *924*).

VENEZUELA: Caracas (Birschel, Gollmer); "prope coloniam Tovar" (Fendler *946*).

Cuscuta corymbosa microlepis Engelmann

(Pl. IV, fig. 20, *A–E*)

Cuscuta corymbosa microlepis Engelmann, Trans. Acad. Sci. St. Louis **1**: 484. 1859.—Progel in Martius, Flora Brasiliensis **7**: 378. 1871.

Cuscuta corymbosa Ruiz & Pavon, Fl. Peruv. **1**: 69, Pl. 105, fig. *b*. 1798. Not Choisy nor Jussieu.

Flowers about 4 mm. long; calyx about reaching the middle of the corolla tube, ovate-cordate anthers about equal to the filaments; scales about reaching the middle of the tube, dentate or with a few scattered processes.

This variety represents the typical variety for the species. Only one specimen has been seen of it, the specimen from which Ruiz and Pavon's description was probably taken.

Type locality: Peru. *Distribution:* Known only from the type locality.

Specimens examined: PERU: (Herb. Ruiz, taken to represent the type, a fragment in the Engelmann herbarium; herb. Pavon, apparently of the same collection as the foregoing. A specimen in the Boissier herbarium without indication of locality or collector is evidently the same).

22. *Cuscuta prismatica* Pavon

(Pl. III, fig. 14, *A–E*)

Cuscuta prismatica Pavon Mss. ex Choisy, Mém. Soc. Phys. Hist. Nat. Genève **9**: 182, Pl. 3, fig. 2. 1841; and in De Candolle, Prodromus **9**: 457. 1845.—Engelmann Trans. Acad. Sci. St. Louis **1**: 485. 1859.

Flowers about 6 mm. long, subsessile, in dense clusters; calyx deep, reaching to the middle of the corolla tube, slightly angled, papillate, red, lobes unequal, much shorter than the calyx tube, ovate, acute, overlapping; corolla cylindrical, granulate, lobes about one fourth as long as the tube, upright to spreading, oblong, acutish and sometimes cuspidate; oval anthers sessile; scales short, not reaching to the middle of the tube, sparingly fringed toward the apex with few processes, bridged below the middle; styles slender, about twice as long as the globose ovary. Only a fragmentary capsule was seen, but that evidently circumscissile. No seeds were seen.

Choisy pictures this species as without scales and Engelmann so describes it, but in all of the flowers opened I never failed to find scales.

Type locality: Guayaquil, Ecuador. *Distribution:* Known only from the vicinity of Guayaquil, Ecuador.

Specimens examined: ECUADOR: Guayaquil (Pavon, the type, a fragment in the Engelmann herbarium; Remy in 1856).

Subsection LEPTILOBAE Engelmann

KEY TO THE SPECIES

Corolla lobes shorter than the tube; flowers commonly reddish	23. *C. partita.*
Corolla lobes ordinarily as long as, or exceeding, the length of the corolla tube	24. *C. umbellata.*

23. *Cuscuta partita* Choisy

Cuscuta partita Choisy, Mém. Soc. Phys. Hist. Nat. Genève **9**: 284, Pl. 5, fig. 3. 1841; and in De Candolle, Prodromus **9**: 460. 1845.—Engelmann, Trans. Acad. Sci. St. Louis **1**: 487. 1859.—Progel in Martius, Flora Brasiliensis **7**: 386, Pl. 128, fig. 6. 1871.—Yuncker, Ill. Biol. Monogr. **6**: 130, figs. 12, 118, and 156. 1921.

Stems medium. Flowers about 2 mm. long, on pedicels shorter than or exceeding the length of the flower, in loose, umbellate-racemose or more densely compact clusters, reddish, glandular; calyx shorter than or exceeding the corolla tube; lobes ovate-lanceolate, acute to acuminate; corolla globose-campanulate, furrowed or angled lengthwise along the region of the stamen attachment; lobes triangular or sometimes lanceolate, acute to acuminate, spreading or reflexed, shorter than the tube, with the tips sometimes inflexed; stamens shorter than the lobes of the corolla, the oval anthers about equal to the slender filaments, or the filaments somewhat subulate and shorter; scales shorter than the corolla tube or reaching the stamens in some specimens, bridged slightly below the middle, fringed with medium-length processes; stigmas comparatively large, slender styles much longer than the globose ovary. Capsule circumscissile, globose, slightly depressed, carrying the withered corolla about it; seeds roundish, compressed, about 1 mm. long, hilum short, perpendicular.

Type locality: "Hab. in Brasiliâ apud Illheos." *Distribution:* Venezuela, Brazil, and Bolivia, and sparingly in the islands off the coast.

Specimens examined: BOLIVIA: (Weddell *3483*, *3611*).

BRAZIL: (Blanchet *3047*, taken to represent the type, a fragment in the Engelmann herbarium); Paraná, Paranagua (Gardner *2684*); Prov. Piauhy (Gardner *2689*); Matto Grosso, Cuyabá (Riedel *846* in part, mixed with *C. racemosa miniata;* Hoehne *4483;* Lindman *A3481*); São Luiz de Caceres (Hoehne *1048*).

VENEZUELA: Barquisimeto (Pittier *6404*).

24. *Cuscuta umbellata* Humboldt, Bonpland, & Kunth

For the description and synonymy of this species see Yuncker, Ill. Biol. Monogr. **6**: 131, figs. 9, 114, 115, and 149. 1921.

Only specimens of the typical variety have been examined from South America. Engelmann segregated his variety *desertorum* on a specimen collected by Martius and named *C. desertorum* by him in his herbarium. His segregation was based on this specimen's having more rudimentary scales than the other varieties. In examining the type specimen I found that the scales were fully developed in the flower examined, and am considering this variety the same as the typical variety.

Specimens examined: BRAZIL: Prov. Ceara (Gardner *2425*); Prov. Piauhy (Martius, the type of *C. desertorum*, a fragment in the Engelmann herbarium).

BRITISH GUIANA: (Jenman *6098*); Georgetown (Rodway; Hitchcock *16564*).

INDEX TO VOLUME IX

(New names and final members of new combinations are in **heavy-face** type.)

Actinomyces and hydrogen-ion concentration, 160
Agaricus, development of: *arvensis*, 512; *campestris*, 509; *comtulus*, 512; *melleus*, 510; *Rodmani*, 512; *tabularis*, temperatures for growth of, 388
Age and maturity of host tissue in relation to virulence of disease, 196
Agropyron, susceptibility of, to disease, 196
Agrostis alba, susceptibility of, to disease, 197
Algal cells, methods of healing in some, 18
Alsine media, viability of seeds in, 268
Alternaria brassicae, 459
Althea rosea, *Puccinia malvacearum* on, 195
Aluminum and iron salts, the growth of field corn as affected by, 47
Amanitopsis vaginata, development of the carpophore of, 511
Amaranthus graecizans, viability of seeds in, 268; *retroflexus*, viability of seeds in, 268
Ambrosia elatior, viability of seeds in, 268
Anthemis cotula, viability of seeds in, 268
Apples, freezing of, in storage, 555
Arenga saccharifera, 82
Arisaema triphyllum and *Arisaema Dracontium*, control of the sexual state in, 72
Armillaria (*mellea*), 510
ARNDT, CHARLES HOMER. The growth of field corn as affected by iron and aluminum salts, 47
Ascochyta pisi, 464
Ash, *Fomes fraxinophilus* on, 506; *Polyporus hispidus* on, 506
Asparagus rust, 194
Aspergillus niger, 459; and hydrogen-ion concentration, 159
Avena sativa, 199

BAILEY, IRVING W. The pollination of Marcgravia: a classical case of ornithophily?, 370
Barley, top and root growth of, 423
BARTLETT, H. H. (See Carl D. La Rue, 79, and Peter J. Klaphaak, 446)
Beal's, Dr. W. J., seed-viability experiment, 266
BENEDICT, R. C. The origin of new varieties of Nephrolepis by orthogenetic saltation II. Regressive variation or reversion from the primary and secondary sports of *bostoniensis*, 140
BENNETT, C. W. (See H. C. Young, 459)
Berberis vulgaris, 199
BERGMAN, H. F. Observations on the effect of water-raking on the keeping quality of cranberries, 245
Betel-nut palm, leaf spot of, 85
Bignoniaceae, stigmas of, 120
Biophysics as a point of view in plant physiology, 37
Birch twigs, temperatures of, 389
Blackberry canes, temperatures of, 387
BONNS, WALTER W. A preliminary study of *Claviceps purpurea* in culture, 339
Bordeaux mixture, the effect of, upon the chlorophyll content of the primordial leaves of the common bean, *Phaseolus vulgaris* L., 535
Bornetia secundiflora, healing in, 18
Botryosphaeria ribis, 386
Botrytis allii, 464; *cinerea* and hydrogen-ion concentration, 161; *cinerea* on strawberries, 204
Brassica nigra, viability of seeds in, 268
Bremia lactucae on lettuce, 233
Bromus secalinus, 187; viability of seeds in, 268
Bursa bursa-pastoris, viability of seeds in, 268

Caeoma abietis-canadensis, origin of pycnia of, 363
Calvatia cyathiformis, temperatures for growth of, 388
Cannabis sativa, sex in, 78
Caracasia, nectaries of, 371
Carbohydrates, effect of, on root growth, 442
Carbon metabolism of the host, 189
Carnation rust, 194

Catalpa bignonioides, stigma of, 100
Cell-plate formation, review of literature of, 296
Cercospora apii, 464; *beticola*, 464
Cestrum nocturnum, 263
Chaetochloa lutescens, viability of seeds in, 268
Chaetomorpha melagonium f. *typica*, healing in, 12
Chara, healing in, 19
Chenopodium album, viability of seeds in, 268
Chlorophyll content of the primordial leaves of the common bean, *Phaseolus vulgaris* L., the effect of Bordeaux mixture upon, 535
Chomiocarpon, 288
Citrus leaves, vein-islets of, 121
Cladophora, healing in, 19
Claviceps Paspali, 340; *purpurea* in culture, a preliminary study of, 339
Cleland, Ralph E. The reduction divisions in the pollen mother cells of *Oenothera franciscana*, 391
Coconut pinnae, incipient drying and wilting as indicated by movements of, 253
Cocos nucifera, leaves used in physiological experiments, 254; Pestalozzia on, 81
Cold storage of fruits and vegetables, 551
Colletotrichum lagenaria, 464; *lindemuthianum*, 459
Conocephalum, 293
Coprinus, certain species of, and *Agaricus campestris*, the origin and development of lamellae in, 509
Coprinus, development of; *atramentarius*, 512; *comatus*, 512; *ephemerus* 513; *micaceus*, 510; *stercorarius*, 513
Corn, the growth of field, as affected by iron and aluminum salts, 47; roots of, with plumule and endosperm removed but with cotyledon attached, effect of increased nitrates upon growth of, 439
Cortinarius pholideus, development of, 512
Cranberries, observations on the effect of water-raking on the keeping quality of, 245
Cronartium ribicola, infection of Ribes by, 197
Culture media, synthetic, growth of some parasitic fungi in, 459
Cultures, plant, the physiological balance in nutrient solutions for, 180
Currant twigs, temperatures of, 386
Cuscuta, revision of the South American species of, 557
Cuscuta acutiloba, 567; *americana*, 573, v. *congesta*, 573, v. *spectabilis*, 573; **argentiniana**, 561; **boliviana**, 565; *bracteata*, 569; **brevisquamata**, 560; *chilensis*, 562; *corniculata*, 570; *corymbosa*, 573, v. *grandiflora*, 573, v. *microlepis*, 573; *epithymum*, 559; *foetida*, 565, v. **pycnantha**, 566; v. *typica*, 566; *globiflora*, 564; **goyaziana**, 568; *grandiflora*, 560; *incurvata*, 571; *microstyla*, 561; *odorata*, 563, v. *botryoides*, 564, v. **Holwayana**, 564, v. *typica*, 564; **orbiculata**, 572; *partita*, 574; *prismatica*, 574; **serrata**, 569; *trichostyla*, 571, v. **carinata**, 572; *typica*, 572; *umbellata*, 575; *xanthochortus*, 567, v. **lanceolata**, 568, v. *typica*, 567
Cytokinesis of Nelumbo, the meiotic, 296

Dactylis glomerata, susceptibility of, to disease, 196
Darlington, H. T. Dr. W. J. Beal's seed-viability experiment, 266
Dianthus sinensis, *Puccinia Caryophylli* on, 193
Digitalis purpurea, stigma of, 102
Diplacus glutinosus, stigmas of, 99
Disease resistance and susceptibility, 183; 215
Dodge, B. O. Studies in the genus Gymnosporangium IV. Distribution of the mycelium and the subcuticular origin of the telium in *G. clavipes*, 354
Dothidella quercus, 464
Drying and wilting, incipient, as indicated by movements of coconut pinnae, 253
Dumortiera irrigua, 288; *trichocephala*, 288; *velutina*, 288
Dupler, A. W. The male receptacle and antheridium of *Reboulia hemisphaerica*, 285

Ecological relations of plants in southeastern Missouri, 1
Elymus, susceptibility of, to disease, 196
Endothia parasitica and hydrogen-ion concentration, 160
Environmental temperatures of fungi in nature, 385
Epilobium hirsutum, root cells of, 123
Erechtites hieracifolia, viability of seeds in, 268

Ergot, the chemistry and toxicology of, 348
Erysiphe graminis, 447; on barley, 426; *Polygoni* on Oenothera, 449
Euonymus japonicus, infected by powdery mildew, 450
Euphorbia maculata, viability of seeds in, 268

Farr, Clifford H. The meiotic cytokinesis of Nelumbo, 296
Fimbriaria, 291
Flax, top and root growth in, 432
Fomes fraxinophilus on ash, 506; *igniarius* on poplar, 506
Forests of southeastern Missouri, 3
Fouquieria splendens, water content of the leaves of, 262
Fragaria sp., temperatures of, 387
Freezing, the effect of low-temperature storage and, on fruits and vegetables, 551
Fungi, environmental temperatures of, in nature, 385
Fusarium and hydrogen-ion concentration, 161; *batatatis*, 464; *conglutinans*, 464; *oxysporum*, 462; *radicicola*, 464

Gericke, W. F. On the physiological balance in nutrient solutions for plant cultures, 180; certain relations between root development and tillering in wheat: significance in the production of high-protein wheat, 366
Gibberella Saubinetii on wheat, 159
Glomerella Gossypii and hydrogen-ion concentration, 159
Growth of field corn as affected by iron and aluminum salts, the, 47
Gymnosporangium, studies in the genus, IV. Distribution of the mycelium and the subcuticular origin of the telium in *G. clavipes*, 354; on *Juniperus virginiana*, 354; *juniperi-virginiana* infections on apple, 197

Hanson, Herbert C. Prairie inclusions in the deciduous forest climax, 330
Hawkins, Lon A. The effect of low-temperature storage and freezing on fruits and vegetables, 551
Healing in some algal cells, methods of, 18
Hendersonia theaecola on tea, 81
Hevea brasiliensis, Pestalozzia on, 79
Hopkins, E. F. Hydrogen-ion concentration in its relation to wheat scab, 159
Hordeum vulgare, absorption of salts by, 316
Hunter, Mabel R. The present status of Scolopendrium in New York State, 28
Hydrogen-ion concentration in its relation to wheat scab, 159

Impatiens aurea, 200
Iron and aluminum salts, the growth of field corn as affected by, 47
Irwin, Marian, and Margaret Weinstein. Comparative studies on respiration XXI. Acid formation and decreased production of CO_2 due to ethyl alcohol, 277

Johnston, Earl S. Undercooling of peach buds, 93
Jungermanniales, 291

Kauffman, C. H., and H. M. Kerber. A study of the white heart-rot of locust caused by *Trametes robiniophila*, 493
Kerber, H. M. (See C. H. Kauffman, 493)
Klaphaak, Peter J., and H. H. Bartlett. A preliminary notice of genetical studies of resistance to mildew in Oenothera, 446

La Rue, Carl D., and H. H. Bartlett. A demonstration of numerous distinct strains within the nominal species *Pestalozzia Guepini* Desm., 79
Lepidium virginicum, viability of seeds in, 268
Lepiota, development of: *clypeolaria*, 512; *cristata*, 512; *seminuda*, 512
Levine, Michael. The origin and development of lamellae in *Agaricus campestris* and certain species of Coprinus, 509
Lignification of mature phloem in herbaceous types, 239
Locust, a study of the white heart-rot of, caused by *Trametes robiniophila*, 493
Lolium temulentum, 234
Lunularia, 293
Lychnis githago, viability of seeds in, 268

Macrosporium brassicae, 459; *commune* and hydrogen-ion concentration, 159; *sarcinaeforme*, 464

Magnolia, cell division in, 302
Malva rotundifolia, viability of seeds in, 268
Marcgravia, the pollination of; a classical case of ornithophily?, 370
Marcgravia cuyuniensis, description of, 377; **purpurea**, description of, 378
Marchantiales, 285
Martynia lutea, stigma of, 103; *proboscidea*, stigma of, 103
Martyniaceae, stigmas of, 120
Meiotic cytokinesis of Nelumbo, the, 296
Mercurialis annua, sex in, 78
MERRIMAN, MABEL L. A new species of Spirogyra with unusual arrangement of the chromatophores, 283
Micrococcus, on *Oenothera Lamarckiana*, 447
Mildew in Oenothera, a preliminary notice of genetical studies of resistance to, 446
Mimulus cardinalis, stigma of, 103; *glabratus*, 108; *glabratus* var. *Jamesii*, 109; *guttatus*, 100; *luteus*, 103; *punctatus*, 117
Mineral nutrients in host, 185
Missouri, ecological relations of plants in southeastern, 1; forests of southeastern, 3
Monoselenium tenerum, 288
MUENSCHER, WALTER C. The effect of transpiration on the absorption of salts by plants, 311

Nelumbo, the meiotic cytokinesis of, 296
Nephrolepis, the origin of new varieties of, by orthogenetic saltation II. Regressive variation or reversion from the primary and secondary sports of *bostoniensis*, 140
NEWCOMBE, F. C. Significance of the behavior of sensitive stigmas, 99
NICHOLS, SUSAN P. Methods of healing in some algal cells, 18
Nicotiana, cell division in, 303
Nitella, healing in, 20
Nitrogen nutrition in host, 185
Norantea anomala, 371; *brasiliensis*, 371
Notoholcus lanatus, 199
Nuphar luteum, cytokinesis in, 297
Nutrient solutions for plant cultures, physiological balance in, 180; solutions for wheat, 270
Nymphaea alba, cell plate in, 298; cytokinesis in, 297

Oenothera, a preliminary notice of genetical studies of resistance to mildew in, 446; *biennis*, 391, viability of seeds in, 268; *biennis Chicago*, 448; *cinerescens*, 449; *franciscana*, the reduction divisions in the pollen mother cells of, 391; *grandiflora*, 391; *Lamarckiana*, infection by Micrococcus, 447; *mississippiensis*, 448; *numismatica*, 448, immunity to mildew, 446; *nutans*, 447; *pratincola*, 448, infection by mildew, 446, hyb. *immunis*, 448, hyb. *viscida*, 448; *Reynoldsii*, 449, immunity to mildew, 446; *scitula*, immunity to mildew, 446; *stenomeres*, infection by mildew, 446
Ornithophily?, the pollination of Marcgravia: a classical case of, 370
Orthogenetic saltation, the origin of new varieties of Nephrolepis by, II. Regressive variation or reversion from the primary and secondary sports of *bostoniensis*, 140

Peach, Elberta, 93; Greensboro, 94; buds, undercooling of, 93
Pears, freezing of, in storage, 555
Pellia epiphylla, 291
Penicillium cyclopium and hydrogen-ion concentration, 161
Peridermium balsameum, origin of pycnia of, 363; *Hydrangeae*, origin of pycnia of, 363; *Peckii*, origin of pycnia of, 363
Peronospora Arthuri, 447
Pestalozzia Guepini Desm., a demonstration of numerous distinct strains within the nominal species, 79; *palmarum*, diseases caused by, 81; *Theae*, on tea, 81
Phaseolus vulgaris L., the common bean, the effect of Bordeaux mixture upon the chlorophyll content of the primordial leaves of, 535
Phleum pratense, susceptibility of, to disease, 197
Phloem, lignification of mature, in herbaceous types, 239
Pholiota, development of; *adiposa*, *flammans*, *squamosa*, 512
Phoma apiicola, 464
Pisum sativum, infection by *Erysiphe Polygoni*, 450
Plagiochasma, 292
Plant physiology, biophysics as a point of view in, 37

Plantago major, viability of seeds in, 268
Plenodomus fuscomaculans, 461
Pluteus admirabilis, development of, 512
Pollination of Marcgravia, the: a classical case of ornithophily?, 370
Polyembryony, 213
Polygonum hydropiper, viability of seeds in, 268
Polyporus hispidus, on ash, 506
Polystigma rubrum, temperatures of leaves affected by, 388
Poplar, *Fomes igniarius* on, 506
Portulaca oleracea, viability of seeds in, 268
Potato, injury from freezing in storage, 555; ovules with two embryo sacs, 213
Prairie inclusions in the deciduous forest climax, 330
Preissia, 288
Puccinia Asparagi, susceptibility of onions to, 196; *Caryophylli*, artificial infection with, 188; *coronata*, 193; *coronifera*, 218, on oats, 190; *dispersa*, 187; *glumarum*, resistance to, in wheat, 186; *graminis* on barberry and wheat, 199; *graminis avenae* on oats, 197; *graminis tritici* on wheat seedlings, 188; *malvacearum* on *Althea rosea*, 195; *poculiformis*, 201; *rubigo-vera* on rye, 198; *secalina*, 218; *Scrghi*, 218, on *Zea Mays*, 190; *triticina*, 199, 218
Pulling, Howard E. Biophysics as a point of view in plant physiology, 37

Radish, respiration in, 277; roots, effect of increased nitrates upon growth of, 438
Raines, M. A. Vegetative vigor of the host as a factor influencing susceptibility and resistance to certain rust diseases of the higher plants I, 183; II, 215
Rank'n, E. J. (See W. E. Tottingham, 270)
Reboulia Charrieri, 286; *hemisphaerica*, the male receptacle and antheridium of, 285; *occidentalis*, 286
Reduction divisions in the pollen mother cells of *Oenothera franciscana*, the, 391
Relations between root development and tillering in wheat, certain: significance in the production of high-protein wheat, 366
Resistance and susceptibility to certain rust diseases of the higher plants, vegetative vigor of the host as a factor influencing, 183; 215
Respiration, comparative studies on XXI. Acid formation and decreased production of CO_2 due to ethyl alcohol, 277; XXII. The effect of lactic acid on the respiration of wheat, 307
Rhamnus cathartica, 199
Rhizoctonia solani, 464
Rhizopus nigricans, 462; on strawberries, 204
Ribes, resistance and susceptibility to infection by *Cronartium ribicola*, 197
Robinia Pseudo-Acacia, white heart-rot of, 493
Root cells of *Salix nigra* Marsh., the relation of age to size in certain, 121
Root growth in seed plants, studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to, 415
Roots of corn, with plumule and endosperm removed but with cotyledon attached, effect of increased nitrates upon growth of, 439; radish, effect of increased nitrates upon growth of, 438
Rumex crispus, viability of seeds in, 268
Rust diseases of the higher plants, vegetative vigor of the host as a factor influencing susceptibility and resistance to certain, 183; 215
Ruth, W. A. The effect of Bordeaux mixture upon the chlorophyll content of the primordial leaves of the common bean, *Phaseolus vulgaris* L., 535
Ruyschia, nectaries of, 371

Salix nigra Marsh., the relation of age to size in certain root cells and in vein-islets of the leaves of, 121
Salts, the growth of field corn as affected by iron and aluminum, 47
Salvia, respiration in, 277
Schaffner, John H., Control of the sexual state in *Arisaema triphyllum* and *Arisaema Dracontium*, 72
Schizophyllum commune, development of, 513
Sclerotinia cinerea, 464; *libertiana*, 464
Scolopendrium in New York State, the present status of, 28; *vulgare*, 28
Scrophulariaceae, stigmas of, 119
Seed plants, studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in, 415

Seed-viability experiment, Dr. W. J. Beal's, 266
Selaginella Kraussiana, 77
Septoria Apii, on celery plants, 233
Sesbania aculeata, floating roots of, 123
Sexual state, in *Arisaema triphyllum* and *Arisaema Dracontium*, control of the, 72
SMITH, EDITH PHILIP. Comparative studies on respiration XXII. The effect of lactic acid on the respiration of wheat, 307
Solanum tuberosum, polyembryony in, 213
Souroubea, 372
Sphaeropsis malorum, 464
Spirogyra, a new species of, with unusual arrangement of the chromatophores, 283; cell wall of, 18; *crassa*, 283; **rectispira**, description of, 284
SPONSLER, O. L. The structure of the starch grain, 471
Starch grain, the structure of the, 471
Sterigmatocystis violae, 464
STEVENS, NEIL E. Rots of early strawberries in Florida and southern California, 204; environmental temperatures of fungi in nature, 385
Stigmas, significance of the behavior of sensitive, 99
Strawberries, rots of early, in Florida and southern California, 204; temperatures of, 387
Strongylocentrotus purpuratus, 282
Susceptibility and resistance to certain rust diseases of the higher plants, vegetative vigor of the host as a factor influencing, 183; 215
Synthetic culture media, growth of some parasitic fungi in, 459
Syringa, rot of, 501

Tecoma radicans, stigma of, 100
TELLEFSEN, MARJORIE A. The relation of age to size in certain root cells and in vein-islets of the leaves of *Salix nigra* Marsh., 121
Temperature, low-, storage and freezing on fruits and vegetables, the effect of, 551; relations to disease virulence, 192
Torenia fournieri, stigma of, 100
TOTTINGHAM, W. E., and E. J. RANKIN. Nutrient solutions for wheat, 270
Trametes robiniophila, a study of the white heart-rot of locust caused by, 493
Transpiration, the effect of, on the absorption of salts by plants, 311
Trauma in relation to susceptibility to disease, 195
TRELEASE, SAM F., Incipient drying and wilting as indicated by movements of coconut pinnae, 253
Trifolium repens, viability of seeds in, 268
Triticum, species of, 199
Tubaria furfuracea, development of, 513
TURNER, THOMAS WYATT. Studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in seed plants, 415

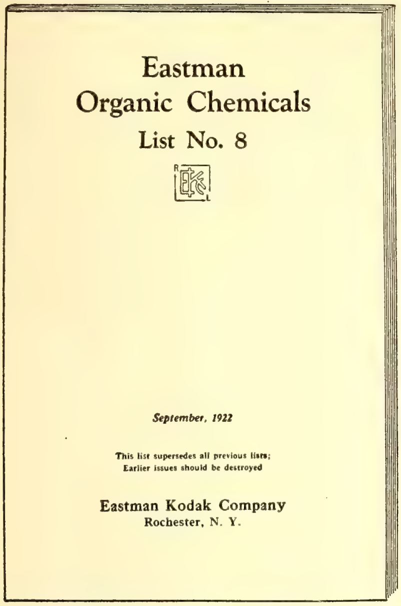
Undercooling of peach buds, 93
UPHOF, J. C. TH. Ecological relations of plants in southeastern Missouri, 1
Uromyces caryophyllinus, 238
Utricularia vulgaris, stigma of, 100

Variation, regressive, or reversion from the primary and secondary sports of *Nephrolepis exaltata* var. *bostoniensis*, 140
Vaucheria, healing in, 19
Vein-islets of the leaves of *Salix nigra* Marsh., the relation of age to size in, 121
Verbascum thapsus, viability of seeds in, 268
Vermicularia sp., 464
Viability of seeds, 266
Vitis vulpina, vein-islets in the leaves of, 121

Water relations to disease virulence, 191
WEINSTEIN, MARGARET. (See Marian Irwin, 277)
Wheat, certain relations between root development and tillering in: significance in the production of high-protein wheat, 366; nutrient solutions for, 270; respiration of, 307; scab, hydrogen-ion concentration in its relation to, 159; seedlings in nutrient solutions, 180
WILSON, CARL L. Lignification of mature phloem in herbaceous types, 239

YOUNG, H. C., and C. W. BENNETT. Growth of some parasitic fungi in synthetic culture media, 459
YOUNG, W. J. Potato ovules with two embryo sacs, 213
YUNCKER, T. G. Revision of the South American species of Cuscuta I, 557

Zea Mays, growth of, 47-71; *Puccinia Sorghi* on, 190, 219; roots of, 123



Eastman
Organic Chemicals
List No. 8

September, 1922

This list supersedes all previous lists;
Earlier issues should be destroyed

Eastman Kodak Company
Rochester, N. Y.

52 Pages Handy Pocket Size. Sent Free Upon Request

Eastman Organic Chemicals

MADE IN U. S. A.

List No. 8

America's Handbook of Organic Chemicals

Over 1400 organic chemicals are listed.
Quality specifications are given for most of the chemicals, example: *Methyl n-Hexyl Carbinol (Free of Ketones)** *B P 178—180°*
All chemicals listed are carried in stock.
Forty-five indicators of P_H ranges from 0—14.3 are available.
Special reagents for inorganic and organic qualitative and quantitative uses are mentioned.
Prices are reduced. Increased demand allows preparation on a larger scale.
Chemists are using List No. 8 as a handbook because of its quality specifications.

There are Eastman Organic Chemicals of specified quality for routine or research work in industry or university

Send for List No. 8

Eastman Kodak Company

Research Laboratory Rochester, N. Y.

WHELDON & WESLEY, Ltd.

Incorporating the old established firms of

JOHN WHELDON & CO.
and
WILLIAM WESLEY & SON

Sole Address: 2, 3, & 4 Arthur Street, New Oxford Street - - LONDON, ENGLAND
Cables: Wheldwesly, London.

BOTANICAL BOOKS AND PERIODICALS
Both New and Secondhand are in stock.

Librarians and Scientists are invited to send their list of Desiderata in
HERBALS AND OTHER EARLY PRINTED BOOKS

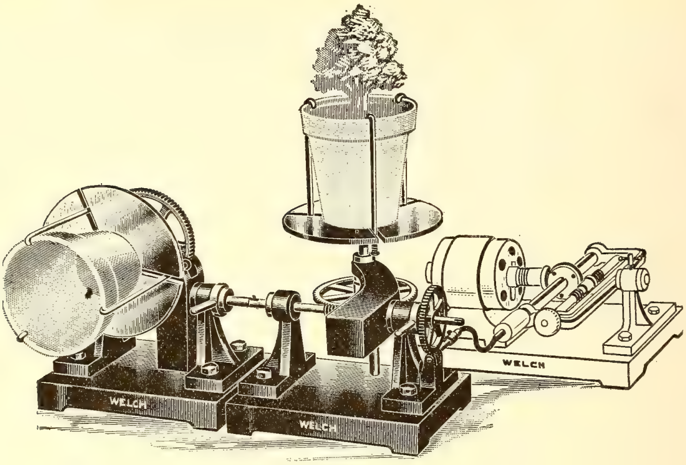
Books on

AMERICAN PLANTS
FOREIGN FLORAS
FERNS, FUNGI
LICHENS, MOSSES
ALGAE, DIATOMS
GARDENING
GARDEN DESIGN

Lists of Publications of the
BRITISH MUSEUM, INDIA OFFICE, AND SMITHSONIAN INSTITUTION
for which Wheldon & Wesley, Ltd., have the Agency, may be had on Application
Write for Catalogues

Always mention the JOURNAL OF BOTANY when answering our advertisements.

CLINOSTAT—Universal—Motor Driven



This outfit consists of two essential parts. First, what is designated as the Master Clinostat and second, what may be designated as the Secondary Clinostat.

The Master Clinostat shown on the right in the illustration, consists of suitable reducing gears arranged so as to reduce motor speed of about 500 R. P. M. down to approximately one revolution in about 15 minutes. This speed is given to the shaft bearing the plate and suitable adjustable clamps hold a flowerpot of 3 or 4 inch size. The supporting plate may be adjusted so that the plant may be rotated in either a vertical or horizontal direction, or in any intermediary direction. There is attached as a driving means a flexible shaft, which is arranged on one end so that it may be inserted in the clutch of the motor rotator, as shown in the illustration. It may also be attached to any other rotator or to any motor.

The Secondary Clinostat consists of a suitable base and reducing gear, and supporting plate with clamp similar to the Master Clinostat and the two shafts engage and thus the Master Clinostat drives the Secondary Clinostat. Any number of these Secondary Clinostats may be thus connected and any number of experiments carried on at the same time. Some of these may be adjusted for rotation in a horizontal direction and some in a vertical direction. The method of engaging the two shafts is such that there is no binding and it is not necessary that they be exactly in alignment.

Each of these instruments is mounted on a heavy iron base, handsomely japanned and all parts are finished in black with nickel plated trimmings.

Cat. No. **8349 Master Clinostat** as described above. Complete with flexible shaft and three clamps. Without flowerpot.

Cat. No. **8349A Secondary Clinostat** as described above. Complete with three clamps but without flowerpot.

We can also supply you with **Herbariums, Plant Presses, Collecting Cases, Dissecting Sets, Microscopes. Specimens, Charts, Lantern Slides, Balances, Glassware, Etc.**

Write for Catalog B—Biological Apparatus and Supplies.

A Sign of Quality QUALITY WELCH SERVICE *A Mark of Service*

W. M. WELCH SCIENTIFIC COMPANY

Manufacturers, Importers and Exporters of
Scientific Apparatus and School Supplies

1516 Orleans Street **Chicago, Ill.**

Professional Advancement Service

THE PROFESSIONAL ADVANCEMENT SERVICE seeks to aid men and women in botany and related subjects in securing such positions as they may desire, and to offer to heads of departments, or directors of laboratories, experiment stations, or other institutions an opportunity of making known the vacancies they seek to fill.

THE METHOD OF THE SERVICE consists in publishing in the American Journal of Botany a brief notice stating the training and experience of the applicant or the requirements of the vacancy. Each person using the Service is designated by number and his identity may be learned on application to the Business Manager of the American Journal of Botany. A list of eligibles and open positions is kept, and inquiries are referred to the advertisers who are mentioned or seem best suited to the requirements stated. The Professional Advancement Service may thus have the opportunity to mention a man for a desirable position or a promotion a number of times during a period of several months. The charge for the service is $2.00 for each case for one year or less. The notice regarding qualifications offered or desired will be published at frequent intervals, but not more than ten times.

Address: The Business Manager, American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

No. 48. Botanist, Ph.D. of five years' standing, with experience in research and teaching, wishes to improve condition. Member technical and honorary societies. Fellow A.A.A.S. Especially interested in physiology and pathology. Several publications. Would like research position or teaching position with sufficient time for research. North central section of the country preferred.

No. 49. Botanist, Ph.D. from a leading university, also from a first rate agricultural college, with thirteen years teaching experience (six in India), desires position, preferably combining teaching with research. Numerous publications, principally ecological. High recommendations. An opening in a tropical or subtropical region is especially desired.

No. 50. Wanted—laboratory instructor in beginning college botany—a young man of good bearing who looks forward to a college or research career in botany and who has perhaps begun graduate work. Preferred that his research interest should be in mycology. Opportunities and prospects good: salary modest.

No. 51. Botanist, student of Pfeffer and Fischer, degrees from the Universities of Zürich (1901), Warsaw (1904), and Moscow (1909). Wide experience as privatdozent; professor and director of botanic laboratory and botanic garden in various institutions in Russia and Tschecho-Slowakia. Thirty-eight published papers in plant physiology, including a text-book of plant physiology of 612 pages. Seeks opportunity for research combined with instruction in an American university or agricultural experiment station. List of publications and further information on request.

No. 52. Man, experienced botanist and teacher, affiliated past seven years with university in large city will make some sacrifice, if necessary, to return to institution in small city or town. Former experience in three other colleges and universities. Correspondence invited.

No. 53. Botanist, B.Sc. from a state university, with broad education in other physical and social sciences, desires position teaching Botany. Two years experience in teaching sciences in a state agricultural school. Holder of a Nebraska State Teachers Certificate. Member Botanical Society of America. Best references.

BROOKLYN BOTANIC GARDEN

MEMOIRS

VOLUME I—DEDICATION PAPERS

CONTAINING 33 CONTRIBUTIONS; PAGES 1–521; PLATES 1–28;
TEXT FIGURES 41

TABLE OF CONTENTS WILL BE MAILED ON REQUEST

The papers contained in this volume were presented April 20–21, 1917, either in person or by title, at the Scientific Program, which formed part of the Dedication Exercises on the occasion of the completion of the Laboratory Building and Plant Houses of the Brooklyn Botanic Garden.

PUBLISHED JULY 6, 1918

Price, $3.50 plus postage. Weight, 4 lbs. Remittance should accompany all orders.
Not offered in exchange

ADDRESS:

THE SECRETARY, BROOKLYN BOTANIC GARDEN
978 WASHINGTON AVE. BROOKLYN, N. Y.

Clarke Memoir Paper

A Permanent Record Paper with Half Tone
and Color Printing Qualities

CLARKE & COMPANY
711 Brunswick Building 225 Fifth Avenue
NEW YORK

THE NEW ERA PRINTING COMPANY

LANCASTER, PA.

Is prepared to execute in first-class and satisfactory manner all kinds of Printing, Electrotyping and Binding. Particular attention given to the work of Schools, Colleges, Universities and Public Institutions

Books, Periodicals
Technical and Scientific Publications
Monographs, Theses, Catalogues
Announcements, Reports, etc.
All Kinds of Commercial Work

Publishers will find our product ranking with the best in workmanship and material, at satisfactory prices. Correspondence solicited. Estimates furnished.

THE NEW ERA PRINTING COMPANY

NATIONAL PHOTO-ENGRAVING CO.

46-48 NEW CHAMBERS STREET

NEW YORK CITY

Telephone; BEEKMAN 3179

PRODUCERS OF HIGH-CLASS ENGRAVINGS
HALF - TONES, LINE PLATES, COLOR WORK, Etc.

Plate-makers to

AMERICAN
JOURNAL OF BOTANY

A TEXTBOOK OF

Mycology and Plant Pathology

New Printing 271 Illustrations 12mo, xiii+779 Pages Cloth $4.00

By JOHN W. HARSHBERGER, Ph.D.
Professor of Botany, University of Pennsylvania

It covers an unusually wide experience in morphology, classification and physiology of the fungi and the advanced cultural and inoculation methods used by the practical bacteriologist and mycologist at the present day. It is the first American book to deal with plant diseases in a general way and to combine an account of specific diseases with general mycology and pathology. The author has brought together much material which, without this work, one must seek in scattered and more or less inaccessible form.

P. Blakiston's Son & Co., Philadelphia

You Will be Interested in

A Naturalist in the Great Lakes Region

By ELLIOT R. DOWNING

A new type of illustrated Nature-guide that will help the student to identify the living things he sees on the earth and in the air and water by showing how Nature, following a definite system, has combined plant and animal life into groups or associations. A book for the hiker's knapsack and the nature-study class.

Educational Edition, cloth $2.50, postpaid, $2.60
De Luke Edition, flexible binding, $3.50, postpaid, $3.60

Methods in Plant Histology

By CHARLES JOSEPH CHAMBERLAIN

This book (third edition, completely revised) contains directions for collecting and preparing plant material for microscopic investigation. Killing and fixing, freehand sectioning, the paraffin method, the Venetian turpentine method, the celloidin method, and the glycerine method are treated in detail, and chapters on making photo-micrographs and lantern slides have been added.

Illustrated, $3.25, postpaid, $3.37

THE UNIVERSITY OF CHICAGO PRESS
5856 Ellis Avenue Chicago Illinois

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1922

President: Henry C. Cowles
University of Chicago

Vice-President: Margaret C. Ferguson
Wellesley College

Secretary: I. F. Lewis
University of Virginia, University, Va.

Treasurer: Irving W. Bailey
Bussey Institution, Harvard University

Councilors:

N. L. Britton
New York Botanical Garden

J. C. Arthur
Purdue University

C. E. Allen
University of Wisconsin

Any one actively interested in botanical work is eligible for membershi Candidates for membership must be recommended by three members, n members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including one subscription to the America Journal of Botany. Candidates proposed for membership, and approve by the Council, may receive one copy of the Journal at $4.00 per year pending their election at the winter meeting of the Society.

Do Our Greenhouses Really Differ So Much From Others

Outwardly most folks are mostly alike.

So are most greenhouses.

But inwardly—that's quite a different matter.

Just as the way a man gives attention to the little things—tells you the kind of a man he is; just so the care and particularity given to the little details in a greenhouse, tells you the kind of a house it is.

Some owners have accused us of being rather fussy about our work, intimating that we could cut corners and get the work done quicker.

And they are right.

But you'll agree that we are also right in refusing to do it.

That then might be one of the differences in which our greenhouses differ.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

PHILADELPHIA
133 So. 12th St.

13

SMITHSONIAN INSTITUTION LIBRARIES
3 9088 00691 0319